STUDIES ON CYTOMORPHOLOGICAL VARIATIONS INDUCED BY CHEMICAL MUTAGENS IN *Capsicum annuum*.

DISSERTATION

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In The Name of Allah, The Most
Gracious The Dispense of Grace and Do
we Not Lend Down from the Clouds
Water in Abundance,
That We May Produce,
Therewith Born and Vegetables,
And Garden of Luxurious Growth?
Al-Quraan (Nabaa; 13-16)

Dedicated

to My

Late. Grand Parents

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Certificate

This is to certify that the dissertation entitled **"Studies on cytomorphological variations induced by chemical mutagens in *Capsicum annuum*"** submitted to the Aligarh Muslim University, Aligarh in partial fulfilment of the requirements for the award of the degree of Master of Philosophy embodies the original research work carried out by **Mr. Mohd. Gulfishan** under my guidance and supervision. The work has not been submitted in part or full for the award of any other degree or diploma of this or any other University.

(Prof. Ainul Haq Khan)
Supervisor

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(Mohd Gulfishan)

CONTENT

LIST OF TABLES

Chapter-1
INTRODUCTION

CHAPTER-1

INTRODUCTION

Mutation is the sudden and heritable change in the genotype of an organism. Although mutations were known to occur much before de Varies (1901) for the first time used the term mutation for the appearance of new phenotype in Evening primrose (Oenothera lamarkiana).

Mutation is the ultimate source of all genetic changes which provide the raw material for evolution and is a very valuable approach for improvement of characters in plants.

If the mutation occurs due to change in base sequences of genes, it may results in transition, transversion, deletion or duplication. These are called as gene or point mutation. Some mutation may be produced by change in chromosome structure involving several genes, or even in chromosome number, they are termed as chromosomal mutation.

Mutation do occurs in natural population (without any treatment by man), these are known as spontaneous mutation. The frequency of these mutations is generally one in ten laks , i.e. 10^{-6}. Mutation may be artificially induced by the treatment known as induced mutations and the agents producing them are termed as mutagens. Mutagens are of two types.

1.1 Physical Mutagens: Physical mutagens includes the following types of radiations.

I. Ionizing Radiation:

(a) Particulate radiation, e.g., α-rays, β-rays, fast neutrons thermal neutrons.

(b) Non particulate radiation (Electromagnetic radiation), e.g., x-rays, and γ-rays.

II. Non Ionising Radiation: e.g., ultraviolet (UV) radiation.

1.2 Chemical Mutagens: There is a long list of chemicals which are used as mutagens. Detailed treatmens of such chemicals is beyond the scope of this chapter. The chemical mutagens are divided into four groups.

(i) Alkylating Agents, e.g. Ethylmethane sulphonate (EMS) Methylmethane sulphonate (MMS) Ethylene imines (EI). Sulpher mustard, nitrogen mustard and Diethyl Sulphate (DES).

(ii) Base Analogues, eg. 5 Bromouracil, 2 Aminopurine, 5 chlorouracil, etc.

(iii) Acridine dyes eg. Acriflavine, Acridine orange, proflavin, Ethidium bromide etc.

(iv) Others eg. Nitrous acid, Hydroxyl amine, Sodium azide, etc.

The physical mutagens have been used since many decades, whereas the use of chemicals is comparatively recent. Altenberg (1928) showed that the frequency of translocation was increased by radiations. Success with the x-rays were achieved by Stadler (1928) in barley and by Goodspeed (1929) in *Datura* and *Nicotiana*.

1.3 Mutation Breeding: A special method of crop improvement:

The genetic improvement of crop plants for various economic characters through the use of induced mutation is referred to as mutation breeding. It is one of the special methods of crop improvement and is commonly used in self pollinated and asexually propagated species, however, this method is rarely used for genetic improvement of cross pollinated species. Mutation breeding will be rewarding under the following situations.

i. When the desired variability is not found in the cultivated varieties or in the germ plasm of cultivated species.

ii. When a high yielding variety has oligogenic defects such as susceptibility to a disease. Mutation breeding is the best course of line for breeders because induced mutagenesis leads to improvement of such variety without much alteration in the genetic background.

iii. When there is a tight linkage between desirable and undesirable characters, mutation breeding is the best way of overcoming such problems.

iv. When in fruit crops, improvement has to be made without change in the taste and colour of the fruit, it can best be achieved through mutation breeding because mutation breeding causes little change in the genetic back ground of parental material.

v. In those crops where sexuality is absent and generation of variability through recombination process is not possible.

vi. In those species where generation cycle in very long, such as plantation crops, fruit trees, and forest trees, mutation breeding is the short cut way of genetic improvement.

Since genetic variability is essential for any crop improvement programme, the creation and management of genetic variability becomes central to crop breeding. Induced variability is generally obtained by mutagenesis, the most important method of inducing alterations by mutagens in a genotype to enlarge the variability in a shortest possible time and provide good scope for selection. Any agent which can change the base sequence has the potential to produce a mutant or changed organism. Interest in induced mutagenesis has been reviewed in recent years due to the fact that

the mutant organism are the indispensable tool for the science of genetics.

Induced mutations are considered as an alternative to naturally occurring genetic variation that serves as the source of germplasm for crop improvement programmes and also an alternative to hybridization and recombination in plant breeding since mutations gives rise to non-existing variations (Brock, 1970). Induced mutations are usually resorted to create variability not available in the existing high yielding or a superior genotype or to improve one or few specific traits of the genotype without altering the otherwise desirable make up of agronomically important characters.

The success in the plant improvement programme, however, depends basically on controlling and directing the induced mutation process for the production of desired mutations. Only through a careful screeing and selection programme the magnitude of genetic variability induced by different mutagens could be exploited for obtaining the desirable lines.

Mutation breeding has helped to rectify certain specific defects in otherwise acceptable cultivars. Induced mutations increase genetic variabilities for certain characters so that selection is more effective and the probability of getting the desired variability is obviously urgently needed, so that suitable type of crop plants may

be developed. Induction of genetic variability by the use of mutagen has been a regular and highly effective system for crop improvement endevours. (IAEA, 1970).

The existing genotypes of chilli are poor yielder and susceptible to stress conditions, insects, pests, and diseases and it is needless to emphasize that this crop requires further genetic improvement for local adaptation.

1.4 *Capsicum annuum* L.

Capsicum annuum L. is a dicotyledonous flowering plant commonly grown worldwide, with many general names in English, such as hot papper, chili, chili or chile pepper, and as well sweet pepper and bell pepper. Sometimes the plant is just called pepper.

It is usually grown as a herbaceous annual in temperate areas. However, ecologically it is a perennial shrub in tropical areas (which may live few years to a few decades), and it can be grown as a perennial in climate-controlled greenhouses. This species includes the vast majority of the cultivated pungent and non-pungent (sweet) *Capsicum* peppers in temperate as well as some tropical areas. In the species *C. annuum* throughout the world, there is phenotypic diversity in plant habit and especially in shapes, sizes, colours, pungency, and other qualities of the fruits. This horticultural, agricultural and biological diversity has helped to make *C. annuum* globally important.

1.5 Taxonomy and Cytology

Classification

Kingdom	Plantae
Division	Phanerogames
Class	Dicotyledon
Order	Solanales
Family	*Solanaceae*
Genus	*Capsicum*
Species	*annuum*

Capsicum species are diploid, with most having 24 chromosomes (n=x = 12), but with several wild species having 26 chromosomes (n = x = 13) *Capsicum annuum* has 24 chromosomes; usually 2 pairs (or sometimes 1) are acrocentric, and 10 (or 11) pairs metracentric or sub-metacentric.

1.6 Area and Production:

Table 1

Year	Area (Lakh Ha)	Production (Lakh Tonn)
1998-1999	8.3	8.7
1999-2000	9.2	10.2
2000-2001	8.2	9.8
2001-2002	8.5	10.6
2002-2003	8.3	8.5
2003-2004	8.2	12
2004-2005	6.9	11.2
2005-2006	6.2	9.8
2006-2007	7.0	11.6
2007-2008	7.2	12.5

India is the largest producer and consumer of chilli among other major producers in the world. India contributes about 36% to the total world production, and is No. 1 in terms of international trade, exporting 20% of its total production. From Table 1, we can infer that since the last decade, chilli production in India is moving northwards on increasing demand from diversified sectors and changing consumption patterns. Dry chilli production rose by nearly 43% from 8.7 lakh tonnes in 1998-99 to 12.5 lakh tonnes in 2007-08. The cardinal factors driving this significant increase in

production are the use of high yielding hybrids in place of varieties, increase in average yield from 1035 kg/ha to 1736 kg/ha, favourable weather conditions, and changing consumption pattern.

In 2007-08, the total acreage brought under chilli cultivation is around 7.2 lakh ha, an increase from last year's 7 lakh ha. Rising export demand coupled with higher price realization in the domestic market have motivated farmers to bring more area under chilli cultivation. According to the Agriculture Department of India, primary estimates of chilli production were around 14 lakh tonnes on considering the increase in acreage from 7 lakh ha to 7.2 lakh ha in 2007-08 and favourable weather conditions. However, the production target was not achieved due to floods in Andhra Pradesh, a major producing center, at harvesting time, resulting in a sharp decline in production to 12.5 lakh tonnes from the earlier estimates of 14 lakh tonnes. However, production in 2007-08 showed an increment by 2.38% over previous year's 11.5 lakh tonnes. India, being the largest producer, is also the largest consumer and exporter. According to trade sources, domestic consumption accounts for around 9.11 lakh tonners per annum.

In India, chillies are grown in almost all the states throughout the country. Andhra Pradesh is the largest producer of chilli in India and contributes about 26% to the total area under chilli, followed by Maharashtra (15%), Karnataka (11%), Orissa (11%), Madhya

Pradesh (7%). The remaining states contributes nearly 22% of the total area under chilli.

The production of chilli in India is dominated by Andhra Pradesh which bestows 53% to the total production. Karnataka is the second-largest producer, contributing 9% of total production followed by Orissa (6%) , West Bengal (6%), Maharashtra (5%), Madhya Pradesh (4%) and others (17%).

Chilli pepper comprises numerous chemicals including steam-volatile oil, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fibre, and mineral elements. The ripe fruits are especially rich in vitamin C. The two chemical groups of greatest interest are the capsaicinoids and carotenoids. The capsaicinoids are alkaloids that give hot chilli papers their characteristic pungency. The rich supply of carotenoids contributes to chilli peppers' nutritional value and Colour. The extracted capsaicin is used in pain balms, cosmetics, and pharmaceutical industry. Capsanthin is a pigment used for natural coloration in jam and jelly preparations. Extracts of chilli contains vitamins A,C and E and it is used in the preparation of ginger beer and other beverages.

Unlike cereals, the reports on induced mutagenesis in chilli are not very extensive. The information on relative specificity and recovery of mutations of varied magnitude is pre-reqeuisite for practical mutation breeding. Keeping these in view, the present

investigation was carried out on two varieties of *Capsicum annuum* L. i.e. pusa jwala and G_4.

The main objectives of present study are to

1. choose the potent mutagen and standardize effective dose as well as treatment condition for releasing high mutation frequency.
2. evaluate the mutagenic effects on various morphological characters.
3. enhance the genetic variability of various quantitative traits.
4. evaluate the effect of mutagenic toxicity on chromosomal behaviour and
5. to compare the sensitivity of two varieties of *Capsicum annuum* L. viz., pusa jwala and G_4 to the different mutagenic treatments.

Chapter-2
REVIEW OF LITERATURE

CHAPTER-2

REVIEW OF LITERATURE

This chapter deals with the literatural survey of effects of mutagen on different aspects of *Capsicum annuum L.* and other related crops with an aim to understand the uses of inducing mutations in crop plants. It includes the extensive research work done by different workers in mutation breeding programmes.

Scientist working on different facets of mutagenesis, since they have been able to accomplish a significant break through in basic understanding of the mechanism of mutagenesis and also its applied value for the benefit of man. Recent decades have witnessed an intensive work on the induction of mutations by radiations, chemicals and other mutagenic agents in plant breeding programme. As per recent information 2252 new mutant varieties have been released belonging to 163 species and spread over 62 counties (IAEA, 2000), and in India 326 mutant varieties in different crops species have been released (Chopra, 2005).

The effect of various physical and chemical mutagens has been extensively studied in *Capsicum annuum* L. and various other related crop plants at two levels.

- Biological damage and Economic characters
- Cytological studies.

2.1 Biological damage and Economic characters:

There are many reports to demonstrate the effects of mutagenic treatments on germination percentage and plant survival. Seed germination and survival rate steadly decreased with increasing dose of two organophosphorous pesticides, Ekalux EC 25 and Metasystox on *Capsicum annuum L.* var. X235 and same result was shown by fungicide "Bavistin and Deltan" (Parakash *et al.,* 1988).

Gradual reduction in germination and survival in *Capsicum annuum L.* was observed when treated with two insecticides "BHC and Nuvacron" and similar results were also obtained shown by herbicides "Lasso and Basagran" treatment (Reddy and Rao, 1981 and 1982a).

The effect of gamma rays and EMS on the seeds of *Capsicum annuum* L. has been studied by Asha and Nayar (1986) who observed an increase in Pollen sterility with increase in dose, and that gamma rays induced a higher percent of sterility as compared to EMS. The cytomorphology of the spontaneous triploid in *Capsicum annuum* L. showed delayed growth and prolonged flowering. It showed marked difference in fruit size and in the ratio of healthy and sterile seeds. (Chennaveeraiah and Habib, 1973).

Raghuvanshi and Joshi (1964) observed delayed and extended flowring with larger and varied number of floral parts in

the colchiploids of *Capsicum frutescence*. Total sterility was observed in the fruits of *Capsicum annuum* produced in the radiation induced polyploids (Indra and Abraham 1977). Pal *et al.* (1941) obtained fertile polyploid with larger fruits in the colchincine induced polyploids of *Capsicum annuum L.* The high Pollen sterility was found in *Capsicum annuum* due to meiotic abnormalities (Novak and Betlach, 1970)

Lakshmi and Nalini (1989) isolated tertiary trisomic in *Capsicum annuum L.* and found phenotypic variations in the height, internodes and leaves. This trisomic had 2n=25 chromosomes. Sadanandam and Subhash (1985) isolated an aneuploid of *Capsicum annuum L.*, var. pusa jawala, followed by 40 kR gamma radiation. The variant was sufficiently vigorous in growth, dark green in colour.

Harini *et al.* (1990) obtained a chromosomal chimaeral plant with three distinct primary branches exhibiting diploids, mixoploids (diploid and tetraploids) and tetraploid condition. It was recorded for the first time in colchicine treated plants of X235, a local cultivar of chilli. These three branches showed differences in leaves, flowers, fruits and stomatal characteristics. The mixoploid branches displayed intermediate feature of both diploid and tetraploid branches. Further, fertility and yield were high in chimaeral plant as compared to those of diploid and tetraploid sibs.

Mesharam *et al.,* (1981) obtained a plant having spontaneous multiple translocation from normal populations of chilli cultivars CA-960. The plant was very healthy having broad green leaves and big size flowers.

Edwin and Reddy (1993) studied the effect of gamma rays, EMS and their combination treatments in hexaploid triticales. Reduction in germination, seedling survival and seedling height was observed in the treated population. Combinations treatments were comparatively more effective followed by EMS treatments. An increase in chlorophyll variants, seedling injury, Pollen sterility, abnormal stomata were also observed in almost all mutagenic treatments.

Jayabalan and Rao (1987) studied the effect of gamma rays, ethyl methane sulphonate and nitroso methyle urea on the seeds of tomato cv, Co-2 and found decrease in percentage of seed germination and seedling survival decrease with increasing dose/concentration. Reduction in germination, seedling growth and Pollen fertility following mutagenic treatments has also been reported in *Capsicum annuum L.* (Singh *et al.*, 1988).

In recent years, the role of mutation breeding in increasing the genetic variability for polygenic characters in a number of crops have been proved beyond doubt (Khan 1984, Ignacimuthu and

Babu 1993, Solanki and Sharma 1999, Waghmare and Mehra 2000 etc).

Raghuvanshi and Singh (1982) induced mutations in *Capsicum* L. when treated with DES, EMS and HS and isolated five viable mutants in M_2 generations. Laxmi and Gupta (1983) studied the response of different concentrations of EMS on various quantitative characters in M_2 generation in *Trigonella*. A significant gradual decrease in plant height, number of branches per plant, number of Pods per plant, number of grains per pod, pod length , and grain yield per plant was noted with an increase in EMS concentration.

Restaino (1983) isolated the pepper brachytic forms (*Capsicum annuum* L.) with different dose of ethyl methane sulphonate and gamma rays. Two recessive brachytic mutants were isolated from M_4, one was compact with reduced internodes, stems, and branches and other was semi compact with a slight reduction in length of the stem and internodes.

Singh (1987) studied the effect of presoaking and various EMS concentrations in diploid and tetraploid fenugreek on different parameters in M_1 generation viz. germination, survival, Pollen fertility, Pods per plant, plant height, branches per plant, seeds per pod in both dry and presoaked sets. The effects of mutagen on various parameters increased with an increase in dose in both

diploid and tetraploid. Tetraploid was resistant than diploid for pollen fertility, pod length and seeds per pod. Presoaking increased the effect of mutagen in both diploid and tetraploid.

Rao *et al.* (1989) studied the effect of gamma rays, ethyl methane suphonate and nitroso methyle urea (NMU) singly and in combination on *Capsicum annuum L.* in M_2 generation and found that the mean values of most of the quantitative characters were lowered as compared to control and frequency of chlorophyll mutations increased following increased mutagenic treatments. Pollen sterility increased with an increase in dose of gamma rays. However, combination treatments were more effective than single doses.

Jain and Agarwal (1993) studied the changes in morphological characters induced by different concentrations of EMS and MMS in *Trigonella foenum-graecum* and observed gradual decrease in seedling length, plant height internodal length, number of nodes per plant, number of flower per plant, number of pods per plant. number of seeds per pod and 100 seed weight in M_1 and M_2 generation.

Bosland *et al.* (1996) determined the optimal conditions for mutagensis in *Capsicum annuum* L. Seeds of cultivar Keystone resistant giant no. 3 were treated with 0.5%, 10% and 1.5% ethylmethane sulphonate (EMS) and exposed for 3, 6 and 9 hr. at

5°C degree C, 10°C, 15°C, and 20°C. Several unique and interesting mutants were generated. In M_1 generation, seed treated with 1.5% EMS at 20°C for 9 hr. had the lowest germination percentage among 36 treatments, but the observed differences in germination were not significant. They suggested that concentration and duration of seed exposure to EMS could be increased to induce even greater number of mutants.

Waghmare and Mehra (2000) studied the genetic variability induced by gamma rays for quantitative characters in *Lathyrus sativus* L. in M_2 and M_3 generations. Grain yield per plant, number of pods per plant, number of seed per pod and plant height showed a significant increase in variability in both the generations while reduction in variability was observed in days to flowering, days to maturity, number of primary branches, pod length and number of seeds per pod.

Jabee and Ansari (2005) treated the seeds of *Cicer arientinum* L. var. K-850 with Hydrazine sulphate (HS) and observed variations in seed germination, growth, morphology, Pollen fertility, and yield in M_1, M_2 and M_3 generations. Lower doses of HS were more effective and efficient, but followed a declining trend with increasing concentrations of HS in var K-850. Significant dose dependent variations in chromosome behaviour were also reported.

2.2 Cytogical Studies

The mitotic and meiotic behaviour of chromosomes is considered to be the most convenient for evaluating the effect of physical and chemical mutagens. Mutagen induced chromosomal aberrations have been reported by many workers in different plants such as in chilli (Meshram and Hume, 1984; Anis *et al.,* 2000), pea, (Kallo, 1972), fenugreek (Anis and Wani, 1997), broad bean. (Bhat *et al.,* 2005). Most of these workers observed dose dependent increase in the frequency of chromosomal abnormalities with respect to mutagenic treatments.

Katiyar (1978) studied the radiocytological effect of gamma rays in *Capsicum annuum* L. and reported a number of meiotic abnormalities such as stickiness/clumping of chromosomes, altered association, breakage, bridges. unequal separation, laggards and abnormal microspores. An increase in Pollen sterility was also observed with increase in dose.

Rao and Laxmi (1980) studied the meiotic abnormalities induced by gamma rays in *Capsicum annuum L.* The meiotic abnormalities includes stickiness, clumping of chromosomes, univalent multivalents, unequal separations laggard, and non orientation of chromosomes. Similar results were obtained in *Lycopersicon esculentum* Mill var. Pusa ruby, treated with gamma rays (Jayabalan and Rao 1987).

Rao and Kumar (1983) isolated three desynaptic plants in *Capsicum annuum* L. The mutant plant showed reduced chiasma frequency and pollen fertility. Kumar and Rao (1985) isolated a desynaptic mutant in *Capsicum frutescens* cv. Tabasco. The desyneptic mutant showed reduced chiasma frequency and pollen fertility. The number of univalents ranged from 12-24 per cell in dsyneptic mutants.

Sadanandam and Subhash (1984) studied the effect of EMS, DES and SA on chiasma frequency per bivalent and per pollen mather cell of M_1 plants in *Capsicum annuum L.* and observed a reduction in chiasma frequency in all mutagenic treatments compared to their respective control. EMS caused greater reduction in chiasma frequency per cell.

Meshram and Patil (1986) studied the cytological effects of dimethyl sulphate (DMS), ethyl methan sulphonate (EMS) and Acid Juice of Mango (AJM) in *Capsicum annuum* L. and reported chromosome stickiness, univalents, multivalents, chromatin bridges, fragments and micronuclei.

Prakash *et al.,* (1988) studied the effect of two fungicides, Bavistin and Deltan on *Capsicum annuum* L. var-X235 and reported a dose dependent increase in various chromosomal abnormalities namely univalents , multivalents stickiness, non-orientation of chromosomes, laggards and chromatin bridges. The mean chiasma

frequency decreased with increased concentration of mutagens. A decrease in pollen fertility was also recorded with increased concentration of mutagen.

Anis and Sharma (1997) made cytological analysis in treated as well as in control plants of *Capsicum annuum* L. treated with EMS, MMS and SA and observed a reduction in chiasma frequency in all mutagenic treatments as compared to their respective control. EMS caused a greater reduction in chiasma frequency than MMS and SA. Various chromosomal aberrations like clumping and stickiness of chromosomes, univalents, multivalents and fragments were observed at metaphase–I.Irregualr grouping of chromosomes and laggards were also found at anaphase stages.

Anis *et al.,* (1998) induced autotetraplody in *Capsicum frutescens* var. suryamukhi by treating apical growing point with colchicine and observed various meiotic abnormalities such as univalents, multivalents, unequal distribution of chromosomes and micronuclei. These irregularities were the major factor for high sterility of pollen grains in induced tetraploid plants of *Capsicum frutescens*. Kumar and Dubey (1998) studied the effect of gamma rays, EMS and DES on meiosis, pollen and seed sterility and survival percentage in M_1 generation of *Lathyrus sativus*. High frequency of translocations leading to multivalent associations involving varied number of chromosomes were induced in all the treatments.

Anis *et al.,* (2000) studied the effects of EMS, MMS and SA on various cytological parameter in M_2 generation of *Capsicum annuum* L. and recorded a greater reduction of chiasma frequency caused by EMS. Various meiotic abnormalities in M_2 plants included univalents, multivalents, fragments, bridges and laggards were recorded. Pollen sterility was observed high as the concentrations of mutagens increased.

Kumar and Rao (2003) isolated six autotriploids from the progeny of a M_2 line of *Capsicum annuum* L. Autotriploid showed gigantism in respect of leaf stomata, flower and pollen sizes. However, these bear fewer flower, branches, fruits and seeds, besides late flowering as compared to their M_1 progenitor and the control. Univalents, multivalents, unequal separation, micronuclei were the frequent chromosomal anomalies. The mean chiasma frequency, pollen fertility, seed fertility was lower as compared to M_1 progenitor and their control.

Kumar and Rao (2006) isolated fasciated stem mutant in a local cultivar of *Capsicum annuum* L. It was characterized by broad-strap like stem, increased plant height, days to maturity, and pollen sterility. Desynepsis, nondisjunction of chromoshomes, chromosome clumping and stickiness, laggard and bridges were found in some PMCs of the mutant while the normal are devoid of these irregularities.

Singh and Chaudhary (2005) irradiated the dormant, dry seeds of solitary pendent variety (LCA-335) and a clustering erect variety (RHRC-CE) of *Capsicum annuum* L. with gamma rays. Radiation induced meiotic abnormalities were found to be directly proportional to the administered gamma rays does. Altered association and other chromosomal aberrations including chromosome stickiness, clumping, bridges, laggards were reported. Concomitantly, dose dependent increase in the percent pollen sterility was directly proportional to the meiotic abnormalities.

Bhat *et al.,* (2005a) studied the effect of gamma rays, ethylmethane sulphonate (EMS) and methylmethane sulphonate (MMS) on meiotic features and pollen fertility in *Vicia faba* L. It was shown that both the physical and chemical mutagens elicited various kind of chromosomal abnormalities and reduction in pollen fertility. However, the meiotic abnormalities were higher in MMS treatments, followed by gamma rays and EMS suggesting that MMS could be more effective in inducing variability followed by gamma rays and EMS in that crop.

Bhat *et al.,* (2006a) irradiated the seeds of broad been (*Vicia faba* L.) varieties minor and major with different doses of gamma rays i.e 10 kR, 20 kR, 30 kR, and 40 kR. Biological parameters such as seed germination, seedling survival, plant height, days to flowering, pollen fertility, days to maturity and yield per plant were

studied in control and treated plants. Meiotic abnormalities such as chromosome clumping, laggards, univalents, multivalents, bridges, disturbed polarity were recorded in the treated population and were found to be directly proportional to the administered doses.

Kumar and Gupta (2009) induced karyomorphological variations in rhree Phenodeviants of *Capsicum annuum* L. Seeds were treated with 0.5% solution of EMS for 3, 5, and 7 h durations and genetic segregation was closely observed. Many chromosomal anomalies like stickiness, bridges, and multivalents, secondary associations, laggards, and precocious movement were observed in all the 3 durations of treatment. These anomalies showed a dose dependent increase in frequency. The morphological parameters showed a decreasing trend along with the increasing dose. However, with the 7-h dose 3 morphologically variant plants were isolated, which varied in plant height, number of nodes, leaf area, 100-seed weight (g) vigorousness and days to maturity, from other sib plants and also from control plants.

Chapter-3
MATERIAL AND METHOD

CHAPTER-3

MATERIAL AND METHOD

3.1 Materials

3.1.1 Varieties used

The certified, healthy and dry seeds of the two varieties of *Capsicum annuum L.* namely Pusa jwala and G_4 were procured from the Division of Genetics, Indian Agricultural Research Institute (IARI) New Delhi. Both these varieties are well adapted to the agroclimatic conditions of Uttar Pradesh, particularly in the region of experimental side (Faculty of Agricultural Sciences, AMU, Aligarh).

3.1.2 Mutagens Used.

The following two mutagens were used separately. The dose concentration of each mutagen used in the present study in given is the Table 3.

3.1.2.1 Methyl Methane Sulphonate (MMS)

The alkylating agents have been found to be the most potent in a wide array of organisms. Within the alkylating groups, MMS has been found to be a very effective chemical mutagen. Like other alkylating agents, MMS reacts with DNA by alkylating the phosphate groups as well as purine and pyrimidine bases and create a gap between DNA molecule causing mutation. It is a colourless liquid with a molecular weight of 110.3.

3.1.2.2 Diethyl Sulphate (DES)

Besides having two alkylating groups, it acts as monofunctional agents, since each group alkylates separately. It is an electrophilic or an acid, or electron pair acceptor. It therefore can react with bases of DNA and cause disturbance in DNA. DES is highly toxic and suspected carcinogenic agent. It is more efficient than EMS, MMS and EI in relation to lethality only.

3.2 Methods:

3.2.1. Preparation of mutagenic solutions

One percent stock solution of MMS and DES was prepared in the phosphate buffer having the pH value 7.0. From this stock solution required concentrations of MMS DES were prepared by using the following formula.

$S_1V_1=S_2V_2$

Where, S_1 = strength of stock solution

V_1 = Volume of stock solution

S_2 = Strength of desired solution

V_2 = Volume of desired solution

The specificity of action of chemical mutagen depends upon the particular conditions of treatment, the more important of which are temperature, duration of treatment and hydrogen ion concentration. Only freshly prepared solutions of MMS and DES were used because alkylating agents are very reactive, even with

water. Hydrolysis usually gives rise to compounds that are no longer mutagenic, but toxic to biological tissues. This means that the mutagen solution must be prepared just at the time of use and never stored.

3.2.2 Pretreatment

Prior to the mutagenic treatment healthy and uniform sized seeds of both the varieties were presoaked in distilled water for 12 hours at room temperature (25 ± 1^0C) in order to activate the embryo.

3.2.3 Treatment with mutagens

The following concentrations of MMS and DES were used in the present study.

Table 3: Concentrations of mutagens used.

Mutagen used	Concentrations (%)
Control	—
MMS	0.01, 0.02, 0.03, 0.04, 0.05
DES	0.01, 0.02 0.03 0.04, 0.05

After the presoaking period was over, the seeds were kept on the blotting paper so as to remove the small droplets of water adhering to the surface of seeds. Thereafter, the seeds were treated with different concentrations of MMS and DES for 6 hours. One set

of each variety was kept untreated to act as control for comparison with the treated populations.

During chemical mutagenic treatments, intermittent shaking was done throughout the treatment period to facilitate sufficient aeration. For uniform absorption, large quantities of mutagenic solution, approximately three times the volume of seeds were used (Konzak *et al.,* 1965). After the treatment period was over the treated seeds were thoroughly washed in running tap water for 1 hour before sowing to remove the residual effect of the mutagen sticking to the seed coat.

3.2.4 Sowing of the seed in the pots:

In each variety a set of 100 seeds (treated as well as untreated), were sown in the four replicates in the first weak of March. The seedling were transplanted into the field when they were 35-36 days old and attained at height of 20-25 cm with 6-7 internodes. The spacing of the seedling was 30x30cm. Recommended agronomic practices were employed for the preparation of the field, sowing, and subsequent management of population to raise a good crop.

3.3 BIOLOGICAL PARAMETERS

3.3.1 Seed germination

Seed germination determines the percentage of seeds that produced healthy roots and shoots. Data on germination was recorded from the pot experiment. The percent of germination was calculated by the following formula.

$$\text{Germination percentage}(\%) = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

$$\text{Inhibition}(\%) = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

3.3.2 Plant survival (%)

Survival of plants was counted at the time of maturity and following formula was used to calculated percent survival.

$$\text{Survival}\ (\%) = \frac{\text{Number of plants at maturity}}{\text{Number of seeds germinated}} \times 100$$

$$\text{Lethality}(\%) = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

3.3.3 Pollen fertility (%)

The pollen fertility was estimated from fresh pollen samples. The pollen grains were dusted in a drop of acetocarmine on a slide. The pollen grains which took the strain and had a regular outline were considered as fertile, while the empty ones without strain and having irregular shape were considered as sterile. The percent fertility was calculated as follows:

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

$$\text{Pollen sterility(\%)} = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100$$

3.4 Cytological Studies

For meiotic studies, young flower buds from 15-20 randomly selected plants were fixed in freshly prepared Carnoy's fluid (alchohol: chloroform: acetic acid in 6:3:1 ratio) supplemented with crystals of ferric chloride for 24 hours. The material was then washed and preserved in 70% alcohol at 4°C. Meiosis was studied by squashing the anthers in 2% acetocarmine. The slides were made permanent by dehydrated them in n-butyl alcohol (NBA) series followed by mounting in Canada balsam and then slides were kept in incubator at 45°C temperature till drying. After drying the extra amount of Canda balsam remained outside the cover slip was cleaned with xylene. Analysis of various stages of meosis was done from each treatment as well as control at metaphase I/II, anaphase I/II and telophase I/II by studying more than 200 dividing PMCs. The abnormalities were recorded on the basis of variations in structure and behavior of chromosomes as compared to control. The photomicrographs were taken from permanent slides with the aid of "Olympus", photo micrographic unit at the magnificanation of 10x eye piece x 100x objective lense. The number of chiasmata per

cell and per bivalent were estimated in treated as well as control population by scoring 100 PMCs at random at metaphase-I stage.

3.5 Quantitative characters

Studies on the quantitative characters were done from the 15-20 normal looking plants in each treatment as well as control populations. The plants which showed great variation from the control were not included for such analysis. The following eleven quantitative characters were studied in M_1 generation.

3.5.1 **Days to flowering:** Days to flowering were noted as the number of days taken by the plant from the date of sowing till the first flower appeared in the plant.

3.5.2 **Plant height (cm) :** Plant height (in cm) was measured at maturity from the base upto the apex of plant.

3.5.3 **Days to maturity:** Days to maturity were noted as the number of days taken by the plant from the date of sowing to the date of harvesting of the plant.

3.5.4 **Number of fruits per plant:** The average number of fruit per plant was determine at maturity.

3.5.5 **Fruit length (cm):** Twenty fruits were selected randomly from each selected plant and there length was measured and mean length of fruits was determined in each treated populations as well as control.

3.5.6 **Fruit girth (cm):** The girth at the mid position of the twenty randomly selected fruits from each selected plant was measured and the mean value of girth from each selected plant was calculated.

3.5.7 **Total yield per plant (g):** Total yield per plant was the weight of total number of fruits harvested from each plant and the yield of each plant was recorded in grams.

3.6 STATISTICAL ANALYSIS

The observations recorded on days to flowering, days to maturity, number of leaves per plant, and plant height in different treatments and control were subjected to statistical analysis with a view to find out the extent of variations induced by the chemical mutagens and their interrelationship.

2.6.1 Mean

It is the measure of central tendency of distribution and defined as the sum of all individual observations divided by total number of observation recorded, thus

$$\overline{X} = \frac{x_1 + x_2 + \ldots\ldots x_n}{N}$$

$$\overline{X}\,(\text{Mean}) = \frac{\sum X_n}{N}$$

Where, x_1, x_2.....x_n=observations

N = Total number of observations involved

2.6.2 Standard Deviation (SD)

It is the positive square root of the average of sum of squares of deviation of all observations from their means. It is calculated by the following formula:

$$S.D.(\sigma) = \sqrt{\frac{(\bar{x} - x_1)^2 + (\bar{x} - x_2)^2 \text{ - - - } (\bar{x} - x_n)^2}{n}}$$

$$S.D.(\sigma) = \sqrt{\frac{\sum (x - \bar{x})^2}{n}}$$

where,

Σx = sum of all individual observations

$\bar{x}$ = Mean of all observations

n = Number of observations

2.6.3 Standard Error (S.E.)

It is the measure of uncontrolled variations present in a sample. It is estimated by dividing standard deviation by the square root of the total number of observations in the sample.

$$S.E. = \frac{SD}{\sqrt{N}}$$

Where,

N= Number of observations

S.D. = Standard deviation.

3.6.4 Coefficient of Variation (C.V.%)

It is the measure of relative magnitude of variation present in the observations relative to magnitude of their arithmetic mean. It

is defined as the ratio of standard deviation to arithmetic mean expressed as percentage and is a unitless number. It computed by applying the following formula:

$$C.V(\%) = \frac{S.D}{\bar{X}} x100$$

Where,

S.D. = Standard Deviation

$\bar{X}$ = Arithmetic Mean

Chapter-4
EXPERIMENTAL RESULTS

CHAPTER-4

EXPERIMENTAL RESULTS

4.1 Studies in M_1 generation:

The effect of Methyl methane sulphonate (MMS) and Diethyl sulphate (DES) was evaluated on seed germination, plant survival, pollen fertility meiosis and various quantitative characters in M_1 generation of *Capsicum annuum L.* var. pusa jwala and G_4

4.1.1 Seed Germination:

Data recorded on the seed germination is shown in table 3. The seed germination decreased with an increase in concentration of the mutagens in both the varities. The maximum decreased in germination was observed at the highest concentration of both the mutagens. In case of MMS treatments, the maximum inhibitory effect on germination i.e,27.65% and 25.00% was observed at 0.05% in var. pusa jwala and G_4 respectively. In case of DES treatments the maximum inhibition was 30.85% and 25.00% at 0.05% in var. pusa jwala and G_4 respectively. The highest inhibitory effect on seed germination i.e., 30.85% was recorded at 0.05% DES treatment in var. G_4.

4.1.2 Plant survival:

The data recorded on plant survival is shown in table 3. The survival of seedling in both the varieties decreased with an increase in concentration of both the mutagenic treatment. The maximum survival percentage was observed at the lowest treatments (0.01% MMS and DES) and the minimum survival percentage was observed at 0.04% of DES in var. pusa jwala. In MMS treatments, the highest lethality (34.5% and 27.37%) was observed at 0.05% in var. pusa jwala and G_4 respectively. In case of DES treatments, the highest lethality (36.64% and 26.25%) was observed at 0.04% and 0.05% concentration in var. pusa jwala and G_4 respectively.

4.1.3 Pollen fertility:

Pollen fertility was decreased with increasing concentration of mutagens in both the varieties (table 3). The maximum pollen fertility i.e., 87.90% and 86.10% was recorded at 0.01%of DES in var.Pusa jwala and G_4 respectively. The minimum pollen fertility i.e.,67.55% and 71.85% was recorded at 0.05% concentration of MMS in pusa jwala and G_4 respectively. The pooled mean values of both pollen fertility and its reduction indicated that MMS treatments were more effective as compared to DES.

4.1.4. Studies on quantitative characters:

The effect of MMS and DES treatments was studied on seven quantitative characters viz., days to flowering, days to maturity, Plant-height, number of fruit per plant, fruit length, fruit girth and total yield per plant in M_1 generation. The data were statistically analysed to find out mean ($\overline{X}$), standard deviation (S.D. %) and coefficient of variation (C.V.%) for these quantitative characters in the treated as well as control populations . Data recorded on these quantitative characters in two varieties of *Capsicum annuum L.* are presented in the tables 3-10.

In the present investigation the mean values of all the quantitative characters shifted in both positive as well as negative directions. The lower concentrations of both the mutagens were more effective in inducing positive shift in mean values for all the quantitative characters except days to flowering and days to maturity where the reverse was true. For fruit girth positive as well as negative shift in mean values was found irrespective of lower or higher concentrations of mutagens.

The pooled mean values for days to flowering was slightly decreased in var. pusa jwala treated with MMS and DES, while in var. G_4 pooled mean values increased when treated with MMS and slightly decreased in DES treated population in comparison to control. Pooled mean values for days to maturity, showed slight delaying effect in both

the varieties treated with MMS and DES, as compare to control. Pooled mean values for plant height showed slight increase in both the varieties treated with MMS, while it was slightly decrease when treated with DES. Pooled mean values for number of fruits per plant decreased in both the varieties except in variety G_4 treated with MMS where, a slight increase in pooled mean value was observed. Pooled mean values for fruit length and fruit girth showed slight increase in both the varieties while total yield per plant showed slight decrease in both the varieties treated with both the mutagens.

The polygenic variability measured in terms of coefficient of variation (C.V.%) increased in all the mutagenic treatments as compared to control in both the varieties. The maximum coefficient of variation was recorded for number of fruits per plant followed by total yeild per plant in both the varieties whereas, the lower values of coefficient of variations were recorded for days to maturity in both the varieties. A perusal of the data recorded for these quantitative character showed that they were affected individually by the mutagens and the differential varietal response to different mutagens was also observed.

4.1.5. Cytological observations:

The control plants of var. pusa jwala and G_4 showed twelve bivalents (2n=24) at diakinesis (Plat-I Fig. 1) and metaphase I (Plate-I Fig. 2) Microsporogenesis in plants raised from treated seeds was highly disturbed. The meiotic studies showed almost similar types of chromosomal aberrations in both the varieties but the frequencies of these aberrations were different (Table-11 and 12).

4.1.5.1 Chromosomal aberrations at metaphase I and II:

The chromosomal aberrations observed at metaphase I/II were univalents, multivalents, stickiness, stray bivalent and precocious separation of chromosomes. (Plate-I-III Fig. 1-12)

(i) Univalents

Univalents were observed at metaphase I in both the mutagenic treatments in var. pusa jwala treated with 0.01% of DES. The frequency of PMCs ranged from 0.37% - 1.66% and 0.72% – 1.96% in var. G_4 treated with DES and MMS respectively. Whereas, it ranged from 1.25% - 3.72% and 1.36% – 2.38% in var. pusa jwala treated with MMS and DES respectively. The maximum frequency of PMCs with univalents was recorded at the highest concentration of each individual treatments except 0.04% DES in var. G_4. The maximum frequency of PMCs with univalents was 3.72% and1.96% at 0.05% MMS in var. pusa jawala and G_4 respectively. (Table 11and 12)

(ii) Multivalents

Multivalents such as trivalents, tetravalents, pentavalents were observed at metaphase I in the treated populations of both the varieties (Plate-I Fig. 5) Multivalents showed dose dependent increase in all the mutagenic treatments in both the varieties. The frequency of PMCs showing multivalents ranged from 0.83% - 2.80% and 0.41%-2.27% in var. pusa jwala, treated with MMS and DES respectively. Whereas, in var.G_4 it ranged from 1.09%-2.74% and 0.74% - 2.50%.in var. G_4 treated with MMS and DES respectively. The maximum frequency of multivalents was 2.80% and 2.74% in var. pusa jawala and G_4 respectively.

(iii) Stickiness

Stickiness or clumping of chromosomes at metaphase I/II was one of the most common meiotic aberrations. The frequency of PMCs showing such aberrations ranged from 1.25%-4.65% in MMS and 0.83-1.90% DES in var. pusa jwala, while it ranged from 1.45%-3.45% in MMS and 1.11%-, 2.08% in DES, in var. G_4.The highest concentration of each treatment showed the higher frequency of PMCs with stickiness of chromosomes in both the varieties. The maximum frequency of PMCs with stickiness was 4.65% and 3.13% at 0.05% MMS in var. pusa jwala and G_4 respectively.

(iv) Precocious Separation

Precocious separation of chromosomes was also observed at metaphase-I in both the mutagentic treatments except in 0.02% of MMS and 0.02% of DES in pusa jwala and 0.01% DES in var. G_4. The frequency of PMCs showing precocious separation ranged from 1.41%-1.39 in MMS, and 0.45% 1.42% in DES treated population of var. pusa jwala whereas it ranged from 0.36%-1.96% in MMS, and 0.40% - 1.66% in DES treated population of var. G_4. The highest concentration of both the mutagenic treatments showed higher frequency of PMCs with precocious separation in both the varieties except at 0.04% DES in var. pusa jwala where, it was maximum in var. pusa jwala treated with 0.04% DES.

The maximum frequency of PMCs with precocious separation was 1.39% in MMS and 1.42% in DES treated population in var. pusa jwala while it was 1.96% in MMS and 1.66% in DES treated population in var. G_4.

(v) Stray bivalent

The frequency of PMCs with stray bivalent was not dose dependent and the stray bivalents were absent in few concentrations of both the mutagen in both the varieties. The frequency of PMCs showing stray bivalent ranged from 0.41%-1.86% in MMS and 0.45% - 1.80% in DES treated population of var. pusa jwala while it ranged

from 0.35% - 1.17% in MMS and 0.40% - 0.78% in DES treated population of var. G_4. The maximum frequency of PMCs with stray bivalent was 1.86% and 1.17% at 0.05% MMS in var. pusa jwala and G_4 respectively.

4.1.5.2 Chromosomal aberration at anaphase I and II.

The chromosomal aberrations found at anaphase I/II were laggard, bridges, with or without fragments, non synchronization and unequal separation of chromosomes. (Plate-III Fig. 5,6 and7)

(i) Laggards

Laggards were observed in both the mutagenic treatments frequency of PMCs with laggards ranged from 0.41% - 2.32% in MMS and 0.90% - 2.72% in DES treated population of var. pusa jwala, while it ranged from 0.35% – 2.35% in MMS and 0.37% - 1.96% in DES treated populations of var. G_4. Frequency of PMCs with laggards was not dose dependent. The maximum frequency of PMCs with laggards was 2.72% in 0.05% DES and 2.35% in 0.05% MMS in var. pusa jwala and G_4 respectively.

(ii) Bridges

The chromotin bridges at enaphse (Plates-II Fig 9) stages were also observed in all the treatments with a few exceptions (0.01% and 0.02% MMS, 0.03% DES in var. pusa jwala, 0.01 MMS and 0.01% DES in var. G_4) The frequency of bridges ranged from 0.43% - 1.39% in

MMS and 0.41% - 1.36% in DES treated var. pusa jwala and 0.70% - 1.66% in MMS and 0.38% - 2.08% in DES treated var. G_4. The Maximum frequency of PMCs with bridges was 1.39% at 0.05% MMS in var. pusa jwala and 2.08% at 0.05% DES in var. G_4.

(iii) Unequal separation

Unequal separation of chromosome at anaphase stages were observed in all the treatments. The frequency of PMCs with unequal separation ranged from 0.41% - 1.86% and 0.83% - 1.88% in var. pusa jwala treated with MMS and DES respectively and 0.36% - 2.74% and 0.78% - 2.50% in var. G_4. treated with MMS and DES respectively. The maximum frequency of PMCs with unequal separation was 1.39% and 2.74% at 0.05% MMS in var. pusa jawala and G_4 respectively. However, a dose dependent increase in the PMCs with unequal separation was not observed.

(iv) Non-synchronization of Chromosome

Non-synchronization of divisional stages was observed in both the mutagenic treatment with a few exceptions (0.04 MMS, 0.03% DES in var. pusa jwala and 0.01%, 0.02% MMS and 0.02%, 0.05% DES in var. G_4). The frequency of PMCs with non -synchronization ranged from 0.41% - 1.39% and 0.45%-1.36% in var. pusa jwala treated with MMS and DES respectively. While it ranged from 1.57%-1.17% and 0.37% - 1.46% in var. G_4. treated with MMS and DES

respectively. The maximum frequency of PMCs with non synchronization was 1.39% at 0.05% MMS, and 1.46% at 0.05% DES in var. pusa jwala and G_4 respectively.

4.1.5.3. Chromosomal aberrations at Telophase I/II

Commonly recorded chromosomal aberrations at telophase I/II were micronuclei, cytomixis and disturbed polarity.

(i) Micronuclei:

Micronuclei were observed in both the mutagenic treatments (Plate III. figs 8) except at 0.03% MMS in var. pusa jawala. The frequency of PMCs with micronuclei ranged from 0.83% - 2.32% and 0.47% - 1.81% in var. pusa jwala treated with MMS and DES respectively and 0.36% - 1.17% and 0.74% - 2.08% in var. G_4. treated with MMS and DES respectively. The maximum frequency of PMCs showing micronuclei was 2.32% at 0.05% MMS and 2.08% at 0.05% DES in var. pusa jwala and G_4 respectively.

(II) Cytomixis

Cytomixis was observed in vary low frequency of PMCs. The frequency of PMCs with cytomixis ranged from 0.41% - 0.94% and 0.45% - 0.94% in var. pusa jwala treated with MMS and DES respectively. While in var. G_4 it was 0.36% - 0.78% and 0.40% - 1.25% treated with MMS and DES respectively. Cytomixis was not those dose dependent. The maximum frequency of PMCs showing

cytomixis was 0.94% at 0.05% MMS and 1.25% at 0.05% DES in var. pusa jwala and G_4 respectively.

(iv) Disturbed polarity

It was a common meiotic abnormality observed at telephase II in all the treatments in both the varieties (Plate-I Fig.11) The frequency of PMCs with disturbed polarity ranged from 0.41% - 2.80% and 0.41% - 0.95% in var. pusa jwala treated with MMS and DES and 0.36% - 1.96%, 0.76% - 2.50% in var. G_4. treated with MMS and DES respectively. The maximum frequency of PMCs with disturbed polarity was 2.80% at 0.05% MMS and 2.50% at 0.05% DES in var. pusa jwala and G_4 respectively.

The results revealed (Table 11 and 12) that meiotic aberrations increased with the increase in concentration of both mutagens in both the varieties. The overall frequency of meiotic aberrations at various stages of meiosis indicated that metaphase aberrations were more common followed by aberrations at anaphase and telophase. However, the frequency of meiotic aberrations was comparatively more in var. pusa jwala than var. G_4.

4.1.5.4 Chiasma frequency

Chiasma frequency characterises pairing of homologous chromosomes at meiosis and controls the degree of recombination besides influencing fertility. It is a constant and genetically controlled

character which does not vary much unless the plants are subjected to any change at genetic level by mutagens or environment. Mutagenic agents are known to bring about changes in chiasma frequency. In the present study the chiasma frequency was calculated at metaphase I in both control and treated populations of both the varieties of *Capsicum annuum L.* (Table 13).

The Chiasma frequency at metaphase-I in var. Pusa jwala (controlled) was 18.6 per cell and in treated populations it ranged from 14.3 to 17.10 and 15.70-18.10 in MMS and DES treated population. Whereas it ranged from 13.30-16.30 and 14.40-16.90 in MMS and DES treated population of var. G4, as compared to control (17.50). The chiasma frequency decreased with increasing concentrations of all the mutagens.

The chiasma frequency per bivalent in var. pusa Jwala treated with MMS ranged from 1.19 to 1. 43 while in DES treated population it ranged from 1.30 to 1.50. The chiasama frequency in control plants of pusa jwala was 1.55.

The chiasma frequency per bivalent in var. G_4 treated with MMS ranged from 1.10-1.35 and in DES treated population it ranged from 1.20 - 1.40 as compared control (1.45). The data showed that MMS cauased more reduction in chiasma frequency then DES in both the varieties.

Table 3. Effect of MMS and DES on seed germination, plant survival and pollen fertility in M_1 generation of *Capsicum annuum* L.var. pusa jwala and G_4.

Treatment %	Germination (%)	Inhibition (%)	Plant Surviwal (%)	Lethality (%)	Pollen fertility (%)	Reduction (%)
			Pusa jwala			
Control	94	-	95.50	-	96.55	-
MMS						
0.01	88	6.38	82.25	13.84	85.20	11.75
0.02	84	10.63	78.90	17.38	80.50	16.62
0.03	78	17.02	75.70	20.73	77.10	20.14
0.04	72	24.04	71.20	25.44	72.60	24.80
0.05	68	27.65	62.50	34.55	67.55	30.03
Pooled Mean	**78**	**17.14**	**74.11**	**22.38**	**76.59**	**20.66**
DES						
0.01	89	5.31	84.50	11.51	87.90	8.95
0.02	82	12.76	80.60	15.60	84.10	12.89
0.03	76	19.14	75.30	21.15	79.60	17.55
0.04	70	25.53	60.50	36.64	76.80	20.45
0.05	65	30.85	70.25	26.43	70.25	27.23
Pooled Mean	**76.40**	**18.71**	**74.23**	**22.26**	**79.73**	**17.41**
			Var. G_4			
Control	92	-	93.50	-	95.25	-
MMS						
0.01	82	10.86	84.50	9.62	84.65	11.12
0.02	78	15.21	79.25	15.24	82.95	12.91
0.03	76	17.39	77.35	17.27	78.40	17.69
0.04	72	21.73	70.69	24.39	75.60	20.62
0.05	69	25.00	67.90	27.37	71.85	24.56
Pooled Mean	**75.40**	**18.03**	**75.93**	**18.77**	**78.69**	**17.38**
DES						
0.01	84	8.70	82.45	11.81	86.10	9.60
0.02	80	13.04	78.20	16.36	83.20	12.65
0.03	77	16.30	75.80	18.93	80.35	15.64
0.04	75	18.47	71.98	23.01	74.40	21.88
0.05	70	25.00	68.95	26.25	72.90	23.46
Pooled Mean	**77.20**	**16.30**	**75.47**	**19.27**	**79.39**	**16.64**

Table 4. Range, mean ($\overline{X}$), shift in $\overline{X}$, standard deviation (S.D.) and coefficient of variation (C.V.) for plant height in M_1 generation of *Capsicum annuum* L. var. pusa jwala and G_4.

Mutagen (%)	Treatment	Range	$\overline{X}$ ± S.E,	Shift in $\overline{X}$	S.D.	C.V. (%)
			Var. Pusa jwala			
	Control	55.2-61.4	58.01±0.85	-	0.95	1.64
	0.01	61.50-65.6	63.00±0.07	+1.08	1.64	2.61
	0.02	55.40-61.5	58.28±1.01	+0.27	2.26	3.88
MMS	0.03	65.2-73.6	68.34±1.30	+10.33	3.09	4.54
	0.04	51.6-59.2	55.40±1.02	-2.61	2.30	4.15
	0.05	48.1-54.6	50.20±1.52	-7.81	3.41	6.80
Pooled Mean			**59.04±1.11**		**2.54**	**4.39**
	0.01	50.20-57.10	53.16±1.30	-4.85	2.90	5.45
	0.02	57.40-69.2	64.40±0.94	+6.39	2.10	3.26
DES	0.03	57.40-59.20	58.60±1.20	+0.59	2.40	4.09
	0.04	48.30-56.10	53.10±0.99	-4.91	2.20	4.15
	0.05	46.50-48.10	50.60±1.27	-7.41	2.85	5.63
Pooled Mean			**55.97±1.14**		**2.49**	**4.51**
			Var. G_4			
	Control	56.50-64.9	60.20±0.39	-	1.10	1.82
	0.01	64.8-68.00	66.34±0.59	+6.14	1.32	2.00
	0.02	66.00-70.10	67.66±0.84	+7.46	1.90	2.80
MMS	0.03	73.20-76.20	75.46±0.73	+15.26	1.65	2.190
	0.04	53.40-62.10	58.40±0.66	-1.80	1.48	2.54
	0.05	50.10-56.40	53.90±0.78	-6.30	1.70	3.15
Pooled Mean			**64.35±0.72**		**1.61**	**2.67**
	0.01	50.20-571	53.16±1.30	-7.04	2.90	5.47
	0.02	53.90-59.2	56.84±0.94	-3.36	2.11	3.72
DES	0.03	62.30-69.2	62.20±1.17	+2.00	2.86	4.60
	0.04	51.20-57.1	53.78±1.30	-6.42	2.93	5.45
	0.05	52.90-60.2	54.96±1.72	-5.24	3.86	7.03
Pooled Mean			**56.18±1.28**		**2.93**	**5.25**

Table 5. Range, mean ($\overline{X}$), shift in $\overline{X}$, standard deviation (S.D.) and coefficient of variation (C.V.) for days to flowering in M_1 generation of *Capsicum annuum* L. var. pusa jwala and G_4.

Mutagen (%)	Treatment	Range	$\overline{X}$ ± S.E.	Shift in $\overline{X}$	S.D.	C.V. (%)
			Var. Pusa jwala			
	Control	30-36	31.10+0.88	-	2.76	8.88
	0.01	26-24	29.20±0.96	-1.90	3.04	1042
	0.02	28-36	30.00±1.00	-1.00	3.36	11.20
MMS	0.03	30-37	29.10+0.72	-2.00	3.35	11.51
	0.04	32-39	30.10±0.79	-1.00	3.80	12.62
	0.05	33-43	33.40±0.82	+2.30	2.99	14.35
Pooled Mean			**30.36±0.65**		**3.30**	**12.02**
	0.01	28-35	29.10±0.77	-2.00	2.65	9.10
	0.02	26-32	28.70±0.65	-2.40	2.85	9.94
DES	0.03	30-36	30.10±0.64	-1.00	3.40	11.30
	0.04	30-37	30.10±0.87	-1.00	3.90	12.96
	0.05	33-42	35.20±0.68	+4.10	2.17	14.15
Pooled Mean			**30.64±0.72**		**2.99**	**11.49**
			Var. G_4			
	Control	33-40	37.90±0.73	-	2.29	6.04
	0.01	32-39	36.80±0.72	-1.10	2.30	6.25
	0.02	30-36	33.40±0.56	-4.50	2.78	8.33
MMS	0.03	33-44	41.10±1.06	+3.20	3.90	9.50
	0.04	35-44	41.00±0.93	+3.10	4.29	10.46
	0.05	35-45	41.30±0.98	+3.40	5.10	12.35
Pooled Mean			**38.72±0.85**		**3.47**	**9.37**
	0.01	32-36	34.00±0.47	-3.90	2.50	7.36
	0.02	32-38	34.80±0.64	-3.10	2.90	8.311
DES	0.03	33-38	36.50±0.60	-1.30	3.55	9.73
	0.04	33-40	38.30±0.68	+0.40	4.20	10.97
	0.05	34-42	38.90±0.59	+1.00	4.95	12.73
Pooled Mean			**36.50±0.59**		**3.62**	**9.82**

Table 6. Range, mean ($\overline{X}$), shift in $\overline{X}$, standard deivation (S.D.) and coefficient of variation (C.V.) for days to maturity in M_1 generation of *Capsicum annuum* L. var. pusa jwala and G_4.

Mutagen (%)	Treatment	Range	$\overline{X}$ ± S.E.	Shift in $\overline{X}$	S.D.	C.V. (%)
			Var. Pusa jwala			
	Control	115-119	117.4 o ±0.57	-	1.78	1.52
	0.01	113–118	115.30 ± 0.50	- 2.1	1.96	1.70
	0.02	113–119	116.60 ±0.67	- 0.8	2.12	1.82
MMS	0.03	115–120	117.80 ± 0.58	+ 0.4	2.38	2.02
	0.04	118–122	119.10 ± 0.88	+1.17	2.80	2.36
	0.05	120–126	123.00± 0.52	+ 5.6	2.70	2.99
Pooled Mean			**118.36±0.63**		**2.39**	**2.17**
	0.01	115-118	117.30±0.65	- 0.10	2.06	1.76
	0.02	115-119	117.10±0.55	-0.30	1.95	1.66
DES	0.03	116-122	118.30±0.64	+0.90	2.22	1.88
	0.04	118-123	120.10±0.60	+2.70	1.92	1.60
	0.05	120.127	123.60±0.71	+6.20	2.33	1.90
Pooled Mean			**119.28±0.63**		**2.09**	**1.76**
			Var. G_4			
	Control	180-185	180.00±1.16	-	2.68	1.48
	0.01	170-176	173.60±0.82	-6.40	2.60	1.50
	0.02	172-178	174.70±0.64	-5.30	2.90	1.66
MMS	0.03	181-187	184.10±0.90	+4.10	2.85	1.55
	0.04	184-189	185.10±0.72	+5.10	2.69	1.70
	0.05	186-191	188.10±0.56	+8.10	2.88	1.52
Pooled Mean			**181.12±0.72**		**2.78**	**1.59**
	0.01	173-178	174.70±0.55	-5.30	1.88	1.85
	0.02	174-179	175.1±0.45	-4.90	2.60	1.49
DES	0.03	179-184	181.30±0.73	+1.30	2.90	1.59
	0.04	184-190	186.10±0.59	+6.10	2.88	1.54
	0.05	186-191	188.50±0.81	+8.50	2.99	1.69
Pooled Mean			**184.15±0.62**		**2.65**	**1.64**

Table 7. Range, mean ($\overline{X}$), shift in $\overline{X}$, standard deviation (S.D.) and Coefficient of variation (C.V.) for fruit lenth in M_1 generation of *Capsicum annuum* L. var. pusa jwala and G_4.

Mutagen (%)	Treatment	Range	$\overline{X}$ ± S.E.	Shift in $\overline{X}$	S.D.	C.V. (%)
			Var. Pusa jwala			
	Control	6.00-7.50	6.46±0.24	-	0.79	12.22
	0.01	9.00-12.10	10.60±0.44	+4.14	1.42	13.40
	0.02	3.50-5.20	4.22±0.16	-2.24	0.59	13.99
MMS	0.03	9.20-15.10	9.60±1.17	+3.14	1.72	17.91
	0.04	10.90-15.60	12.28±0.99	+5.82	2.12	17.26
	0.05	4.00-7.60	6.15±0.35	-0.31	1.12	18.21
Pooled Mean			**8.57±0.42**		**1.39**	**16.15**
	0.01	4.00-7.60	6.20±0.38	-0.26	1.22	19.60
	0.02	9.90-11.90	10.10±0.27	+3.64	1.25	12.38
DES	0.03	9.60-12.00	10.67±0.23	+4.12	1.30	12.26
	0.04	6.00-7.90	7.25±0.24	+0.79	0.95	13.10
	0.05	4.00-5.90	4.82±0.23	-1.64	0.74	15.35
Pooled Mean			**7.80±0.27**		**1.09**	**14.53**
			Var. G_4			
	Control	4.50-6.20	5.90±0.60	-	0.33	5.59
	0.01	5.30-6.10	5.86±0.09	-0.04	0.35	5.98
	0.02	5.00-6.10	5.53±0.12	-0.37	0.45	8.14
MMS	0.03	4.20-6.20	5.30±0.22	-0.60	0.72	13.58
	0.04	7.40-8.10	7.79±0.67	+1.89	0.48	6.17
	0.05	6.00-6.90	6.48±0.10	+0.58	0.65	10.03
Pooled Mean			**6.19±0.24**		**0.53**	**8.78**
	0.01	5.20-6.90	5.25±0.62	-0.65	0.35	6.66
	0.02	5.90-7.60	6.06±0.70	+0.16	0.85	14.02
DES	0.03	2.90-5.00	4.42±0.11	-1.48	0.75	16.97
	0.04	8.40-10.10	9.27±0.16	+3.37	0.78	8.42
	0.05	5.20-5.90	5.46±0.20	-0.44	0.74	13.56
Pooled Mean			**6.09±0.35**		**0.69**	**11.92**

Table 8. Range, mean ($\overline{X}$), shift in $\overline{X}$, standard deviation (S.D.), coefficient of variation (C.V.) for fruit girth in M_1 generation of *Capsicum annuum* L var. pusa jwala and G_4.

Mutagen (%)	Treatment	Range	$\overline{X}$ ± S.E,	Shift in $\overline{X}$	S.D.	C.V. (%)
		Var. Pusa jwala				sds
	Control	2.30-2.50	2.23±0.05	-	0.17	7.92
	0.01	1.90-2.40	2.22±0.08	-0.01	0.27	12.34
	0.02	2.50-3.10	2.95±0.30	+0.73	0.96	13.56
MMS	0.03	2.10-3.10	2.41±0.10	+0.18	0.34	14.17
	0.04	2.10-2.50	2.20±0.05	+0.03	0.18	08.01
	0.05	2.90-3.60	2.41±0.08	+0.18	0.28	11.65
Pooled Mean			**2.43±0.12**		**0.40**	**11.94**
	0.01	2.10-2.70	2.33±0.06	+0.10	0.22	9.45
	0.02	2.00-3.00	2.43±0.08	+0.20	0.28	11.80
DES	0.03	2.00-2.60	2.20+0.06	-0.01	0.20	08.82
	0.04	2.70-3.50	2.95±0.06	+0.72	0.35	11.86
	0.05	3.10-3.90	3.28±0.07	+1.05	0.30	9.15
Pooled Mean			**2.63±0.06**		**0/27**	**10.21**
		Var. G_4				
	Control	2.50-3.10	2.93±0.09	-	0.21	7.16
	0.01	3.10-3.80	3.39±.0.10	+0.46	0.32	9.43
	0.02	2.90-3.30	3.08±0.06	+0.15	0.25	8.11
MMS	0.03	2.00-2.60	2.24±0.07	-0.69	0.21	9.37
	0.04	3.00-4.10	3.90±0.11	+0.97	0.37	9.49
	0.05	2.10-2.80	2.46±0.07	-0.47	0.25	10.16
Pooled Mean			**3.01±0.08**		**0.28**	**9.31**
	0.01	2.50-3.10	2.63±0.05	-0.30	0.19	7.22
	0.02	2.80-3.40	3.28±0.09	+0.35	0.29	8.84
DES	0.03	3.10-3.80	3.46±0.08	+0.53	0.25	7.23
	0.04	3.70-4.20	3.88±0.05	+0.95	0.35	9.02
	0.05	2.20-2.80	2.43±0.07	-0.50	0.28	11.53
Pooled Mean			**3.13±0.06**		**0.27**	**8.76**

Table 9. Range, mean ($\overline{X}$), shift in $\overline{X}$, standard deviation (S.D.) and coefficient of variation (C.V.) for fruit per plant in M_1 generation of *Capsicum annuum* L. var. pusa jwala and G_4.

Mutagen (%)	Treatment	Range	$\overline{X}$ ± S.E,	Shift in $\overline{X}$	S.D.	C.V. (%)
			Pusa jwala			
	Control	15-22	17.50±0.68	-	2.17	12.40
	0.01	14-20	16.30±0.77	-1.20	2.45	15.03
	0.02	15-22	19.10±0.75	+1.60	2.37	12.40
MMS	0.03	13-20	17.00±0.76	-0.50	2.40	14.11
	0.04	10-17	14.20±0.69	-3.30	2.20	15.40
	0.05	9-15	11.60±0.56	-5.90	1.78	15.34
Pooled Mean			**15.64±0.70**		**2.24**	**14.45**
	0.01	17-29	25.10±1.05	+7.50	3.33	13.3
	0.02	16-22	20.40±0.81	+2.90	2.59	12.60
DES	0.03	15-22	18.20±0.78	+0.70	2.48	13.62
	0.04	9-15	12.10±0.73	-5.40	2.33	19.24
	0.05	9-13	11.10±0.48	-6.40	1.52	13.70
Pooled Mean			**17.38±0.66**		**2.45**	**14.50**
			Var. G_4			
	Control	10-17	13.40±0.80	-	2.54	18.96
	0.01	10-18	15.20±0.90	+1.80	2.97	19.54
	0.02	13-25	19.90±1.06	+6.50	3.38	16.99
MMS	0.03	9-16	11.40±0.90	-2.00	.67	23.42
	0.04	9-14	11.20±0.52	-2.20	1.62	14.47
	0.05	7-12	9.80±0.60	-3.60	1.88	19.18
Pooled Mean			**13.50±0.80**		**2.50**	**18.72**
	0.01	9-16	13.00±0.85	-0.40	2.70	20.76
	0.02	10-18	13.60±0.84	+0.20	2.67	19.63
DES	0.03	10-19	13.10±0.99	-0.30	3.14	23.96
	0.04	8-15	11.90±0.78	-1.50	2.46	20.67
	0.05	6-14	9.90±0.79	-3.50	2.47	24.95
Pooled Mean			**12.30±0.85**		**2.68**	**21.99**

Table 10. **Range, mean ($\overline{X}$), shift in $\overline{X}$, standard deviation (S.D.) and coefficient of variation(C.V.)for total yield per plant (g) in M_1 generation of *Capsicum annuum* L. var. pusa jwala and G_4.**

Mutagen %	Treatment	Range	$\overline{X} \pm$ S.E,	Shift in $\overline{X}$	S.D.	C.V. (%)
			Var. Pusa jwala			
	Control	4.40-6.10	5.14±0.29	-	0.70	13.6
	0.01	4.96-7.50	6.038±0.26	+0.89	0.87	13.90
	0.02	4.00-5.10	4.34±0.14	-0.80	0.65	14.90
MMS	0.03	2.80- 4.00	3.50±0.15	-1.64	0.55	15.72
	0.04	2.60-4.00	2.90±0.15	-2.06	0.47	16.20
	0.05	2.60-3.10	2.10±0.11	-2.31	0.40	19.04
Pooled Mean			**3.77±0.16**		**0.58**	**15.95**
	0.01	3.83-4.57	4.04±0.06	-1.1	0.60	14.86
	0.02	2.80-4.00	5.20±0.12	+0.51	0.80	15.38
DES	0.03	2.50-3.90	3.22±0.15	-1.92	0.55	17.08
	0.04	2.50-3.70	3.23±0.140	-1.91	0.70	21.74
	0.05	2.10-3.00	2.30±0.08	-2.55	0.55	23.92
Pooled Mean			**3.59±0.11**		**0.64**	**18.59**
			Var. G_4			
	Control	3.40-4.30	3.81±0.12	-	0.35	9.18
	0.01	3.50-4.30	3.95±0.08	+0.14	0.45	11.53
	0.02	3.55-4.35	4.00±0.07	+0.19	0.50	12.50
MMS	0.03	3.40-4.00	3.60±0.06	-0.35	0.48	13.34
	0.04	2.90-3.60	3.33±0.07	-0.48	0.55	16.52
	0.05	2.50-3.40	2.80±0.12	-1.01	0.58	20.72
Pooled Mean			**3.53±0.08**		**0.51**	**14.92**
	0.01	3.80-4.95	4.19±0.12	+0.38	0.40	9.55
	0.02	3.95-5.20	4.25±0.12	+0.99	0.50	11.76
DES	0.03	3.90-4.16	3.64±0.13	-0.17	0.44	12.08
	0.04	3.10-3.99	3.20±0.10	-0.47	0.35	14.06
	0.05	2.95-3.20	3.02±0.03	-0.77	0.55	18.21
Pooled Mean			**3.66±0.10**		**0.44**	**13.24**

Table 11. Meiotic abnormalities (%) at different stages of meiosis induced by MMS and DES in *Capsicum annuuum L.* var. Pusa jwala

Concentration	Total No. of PMC'S observed	Metaphase I/II					Anaphase I/II				Telephase I/II			Abnormal PMCs
		Univalents	Multivalents	Stickiness	Stray bivalent	precocious separation	Laggard	Bridge	unequal separation	Non-synchronization	Micronuclei	Cytomixis	Disturbed Polarity	(%)
Control	250	-	-	-	-	-	-	-	-	-	-	-	-	-
MMS (%)														
0.01	240	1.25	0.83	1.25	0.41	0.83	0.41	-	0.83	0.41	0.83	0.41	0.41	7.91
0.02	230	1.73	0.86	1.30	0.43	-	0.86	-	0.86	0.43	0.86	-	1.30	9.13
0.03	240	1.66	1.25	3.75	-	0.41	0.41	0.83	0.41	0.41		0.83	0.41	11.25
0.04	230	2.60	1.73	3.91	1.30	0.86	1.30	0.41	1.73	-	1.30	0.43	1.66	19.13
0.05	215	3.72	2.80	4.65	1.86	1.39	2.32	1.39	1.96	1.39	2.32	0.94	2.86	25.59
														73.01
DES (%)														
0.01	250	-	0.41	0.83	-	0.83	1.25	0.41	0.83	-	1.25	-	0.41	6.80
0.02	230	1.36	0.90	1.80	0.48	-	0.90	0.45	1.36	0.1.5	0.90	-	0.45	8.69
0.03	212	1.88	0.47	1.41	-	0.94	1.41	-	1.88	-	0.47	0.94	-	10.85
0.04	210	2.38	1.42	1.90	0.47	1.42	1.90	0.47	1.42	0.95	1.42	0.47	0.95	15.24
0.05	220	1.81	2.27	1.36	1.80	0.45	2.72	1.36	1.81	1.36	1.81	0.45	0.90	20.45
														62.03

Table-12: Meiotic abnormalities (%) at different stages of meiosis induced by MMS and DES in *Capsicum annuum L.* var. G_4

Concentration	Total No. of PMC'S observed	Metaphase I/II					Anaphase I/II				Telephase I/II			Abnormal PMCs (%)
		Univalent	Multivalent	Stickiness	stary bivalent	precocious separation	Laggard	Bridge	unequal separation	Non synchronization	Micronuclei	cytomixis	disturbed Polarity	
Control	2.50	-	-	-	-	-	-	-	-	-	-	-	-	-
MMS (%)														
0.01	275	0.27	1.09	1.45	0.36	0.36	0.72	-	0.36	-	0.36	-	0.36	6.90
0.02	285	1.05	1.40	1.75	0.35	0.70	0.35	0.70	0.35	-	0.70	0.36	0.35	8.77
0.03	270	0.74	1.48	1.48	0.74	0.37	1.11	0.74	1.12	0.37	1.12	0.74	0.74	11.12
0.04	250	1.20	2.00	2.40	0.80	1.20	1.67	1.66	1.20	0.80	0.80	0.40	1.67	16.00
0.05	225	1.96	2.74	3.13	1.17	1.96	2.35	1.56	2.74	1.17	1.17	0.78	1.96	23.14
DES (%)														
0.01	270	0.37	0.74	1.11	-	-	0.37	-	1.12	0.37	0.74	1.11	1.12	6.67
0.02	260	0.76	-	1.15	-	1.53	0.76	0.38	1.15	-	1.54	-	0.76	8.46
0.03	250	1.20	0.80	1.20	0.40	0.40	1.20	0.40	0.80	0.40	1.60	0.40	1.20	11.20
0.04	255	1.17	1.46	1.96	0.78	0.39	1.96	1.17	0.78	1.46	1.96	-	1.46	15.68
0.05	240	1.66	2.50	2.08	-	1.66	1.66	2.08	2.50	-	2.08	1.25	2.50	21.66

Table13. Effect of MMS and DES on Chiasma frequency at Metaphase-I in M_1 generation of *Capsicum annuum* L. var. pusa jwala and G_4.

Treatment	Var. Pusa jwala		Var. G_4	
	Chiasmata per cell	Chaismata per bivalent	Chiasmata per cell	Chaismata per bivalent
Control	18.60	1.55	17.50	1.45
MMS(%)				
0.01	17.10	1.43	16.30	1.35
0.02	16.80	1.40	15.70	1.30
0.03	16.10	1.35	14.30	1.19
0.04	15.20	1.27	13.90	1.15
0.05	14.30	1.19	13.30	1.10
DES (%)				
0.01	18.10	1.50	16.90	1.40
0.02	17.80	1.48	16.10	1.34
0.03	17.20	1.43	15.70	1.30
0.04	16.50	1.37	15.20	1.26
0.05	15.70	1.30	14.40	1.20

Table- 14: Frequency (%) of morphological variant induced by MMS and DES in M_1 generation of *Capsicum annuum L.* var. pusa jwala and G_4.

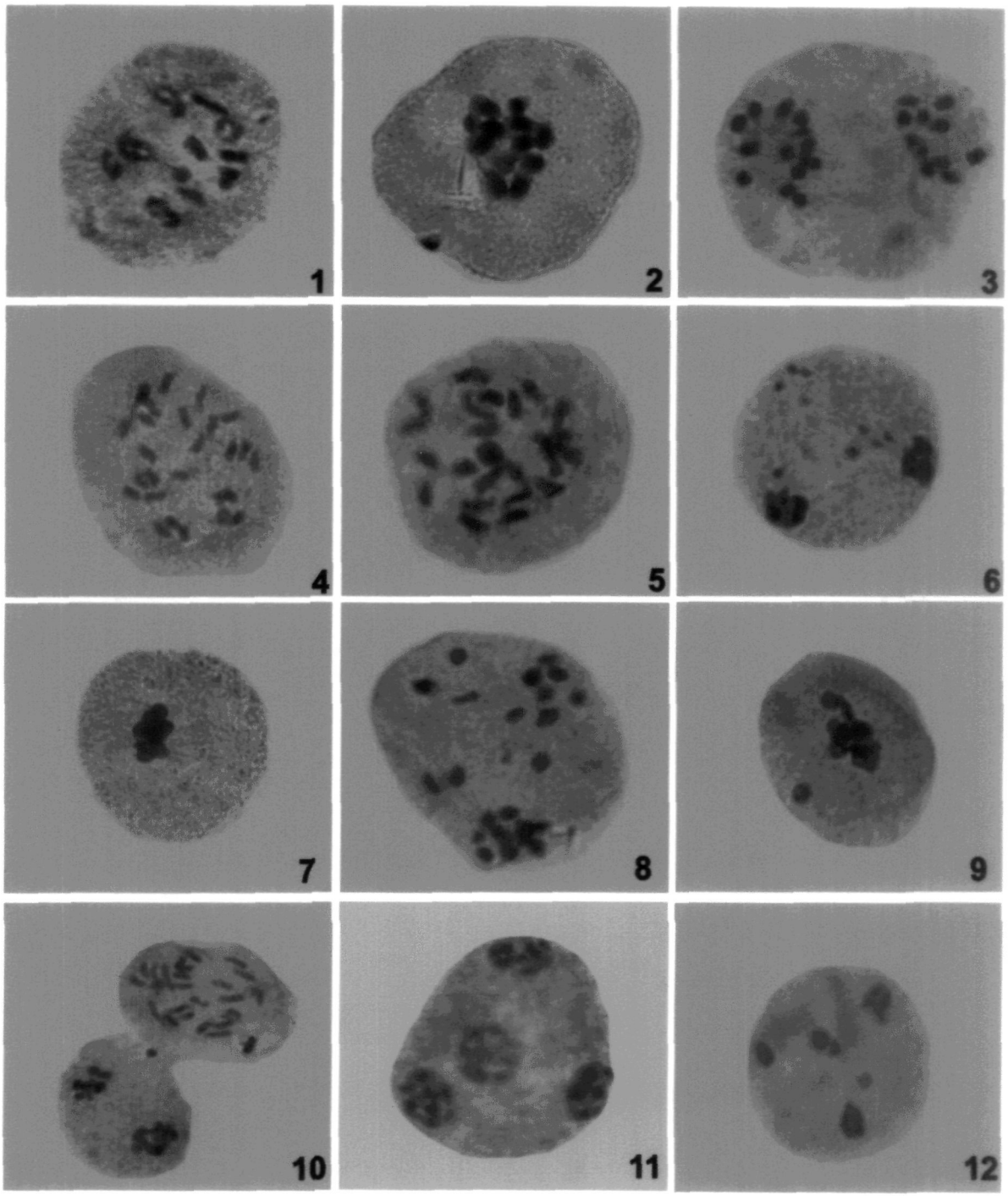
Variants	Pusa jwala		G_4	
	MMS	**DES**	**MMS**	**DES**
Tall	0.55	0.45	0.45	0.65
Dwarf	0.49	0.35	0.20	0.20
Bushy dwarf	0.30	0.25	-	0.20
Long fruit	0.77	0.65	0.55	0.66
Short and thick fruit	0.39	0.45	0.48	0.36
High yielding	0.50	0.39	0.65	0.40
Total	**3.49**	**2.54**	**2.63**	**2.59**

EXPLANATION OF FIGURES

Plate-I

1. : PMC showing 12 bivalents at diakinesis (control) var. pusa jwala.
2. : PMC showing 12 bivalents at metaphase (control) var. pusa jwala.
3. : PMC showing unequal separation (13:11) at anaphase-I. (0.03 DES)
4. : PMC showing delay in disjunction of bivalent at early anaphase-I. (0.05MMS)
5. : PMC showing 11-univalent, 3-bivalent, 1-trivalent and 1-quadrivalent. (0.04DES)
6. : PMC showing laggards at anaphase-I. (0.01MMS)
7. : PMC showing sticky metaphase. (0.03DES)
8. : PMC showing laggards at anaphase-I. (0.02DES)
9. : PMC showing stray bivalent at sticky metaphase. (0.03MMS)
10. : PMC showing migration of chromosomes from one cell to another. (0.05MMS)
11. Telophase-II showing linear arrangement of 3 nuclei due to disturbed polarity. (0.03MMS)
12. PMC showing disturbed polarity at anaphase-II with laggards. (0.04DES)

Plate - I

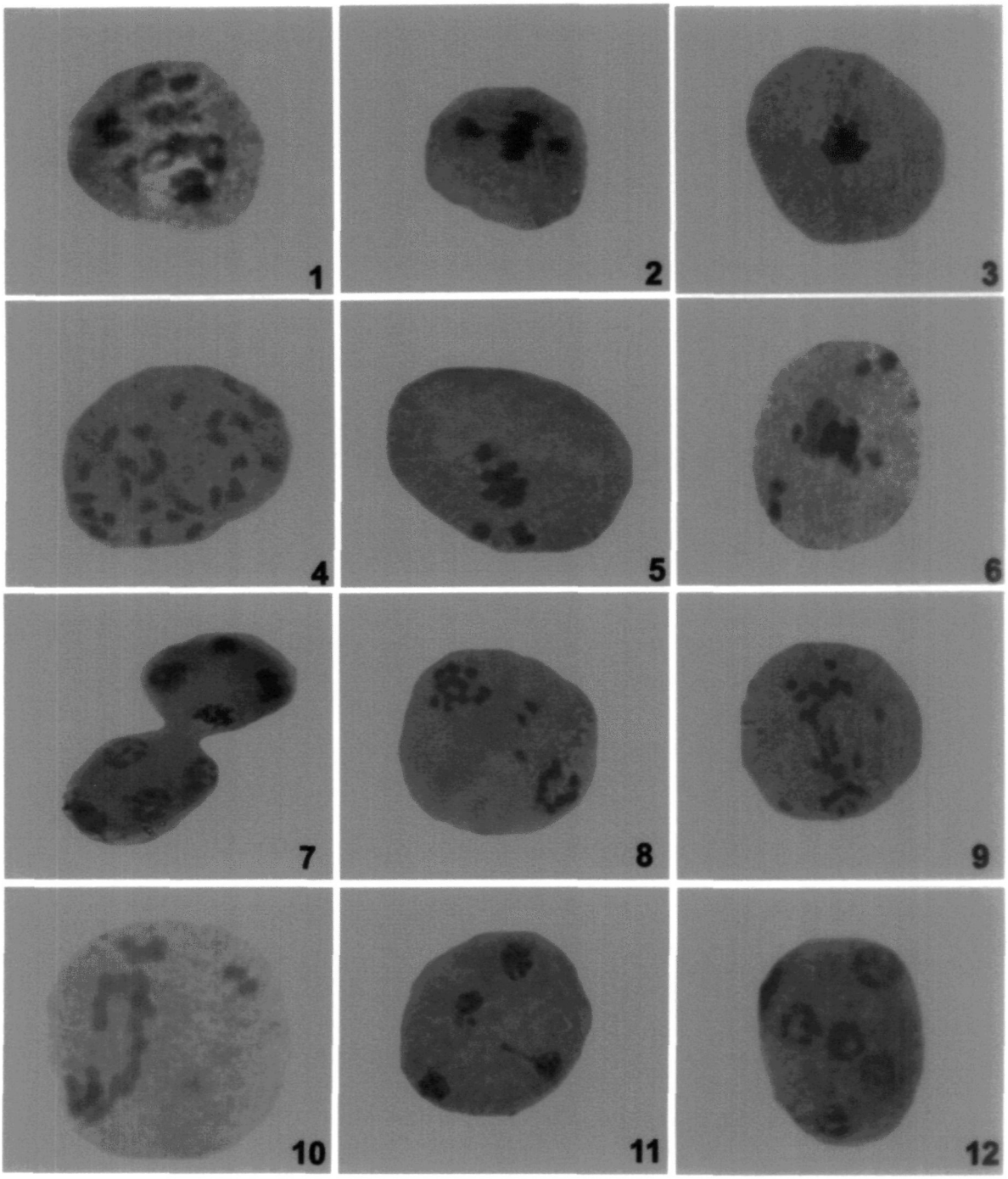


EXPLANATION OF FIGURES

Plate-II

1. : PMC showing 12 bivalents at diakinesis (control) var. G_4.
2. : PMC showing three groups of sticky chromosomes at metaphase-I. (0.01MMS)
3. : PMC showing sticky metaphase-I. (0.01MMS)
4. : PMC showing unequal distribution of chromosome (8:16) at anaphase-I. (0.04MMS)
5. : PMC showing stray bivalent at sticky metaphase-I . (0.02DES)
6. : PMC showing precocious separation at metaphase-I. (0.03DES)
7. : PMC showing cytomixis by direct fusion of the cell at telophase-II. (0.04DES)
8. : PMC showing anaphase-I with unequal distribution (9:12) and laggards (0.03DES)
9. : PMC showing thick sticky bridge with laggards at anaphase-I. (0.03MMS)
10. : PMC showing sticky bridge at anaphase-I. (0.05MMS)
11. : PMC showing telophase-II with broken chromatin bridge and fragment. (0.03MMS)
12. : PMC showing 5 groups of chromosomes at telophase-II. (0.04MMS)

Plate - II

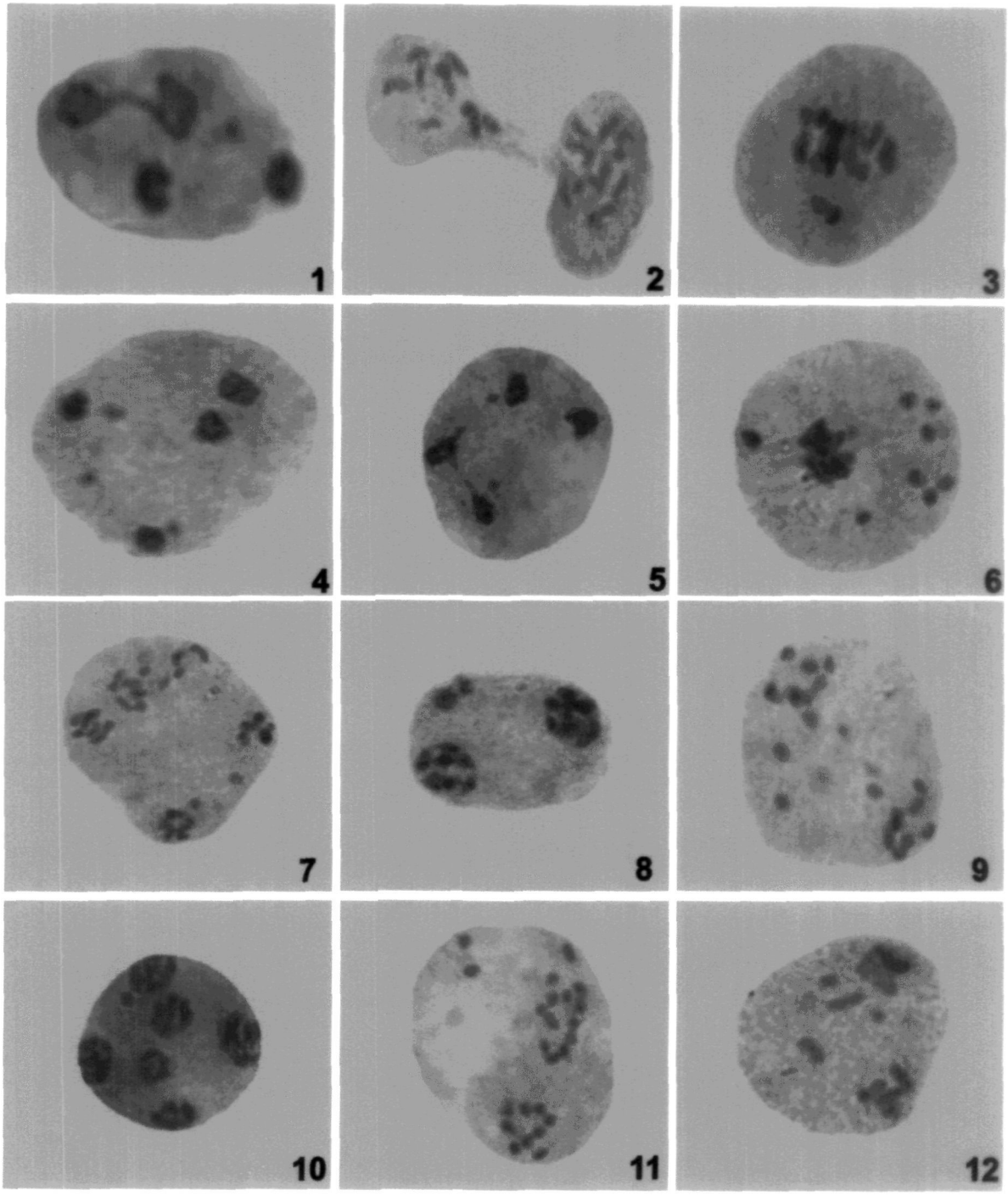


EXPLANATION OF FIGURES

Plate-III

1. : PMC showing bridge at telophase-II with micronuclei (var. pusa jwala). (0.05DES)
2. : PMC showing cytomixis with migrating chromosomes through connecting tube (var. pusa jwala). (0.03MMS)
3. : PMC showing stray bivalent at metaphase-I (var. pusa jwala). (0.01MMS)
4. : PMC showing telophase-II with micronuclei (var. pusa jwala). (0.05MMS)
5. : PMC showing bridge with fragment at anaphase-II (var. pusa jwala). (0.04DES)
6. : PMC showing precocious separation at sticky metaphase-I. (var. pusa jwala). (0.02DES)
7. : PMC showing laggards at anaphase-II (var. G_4). (0.04DES)
8. : PMC showing micronuclei at telophase –I (var. G_4). (0.03MMS)
9. : PMC showing laggards at anaphase-I (var. G_4). (0.02MMS)
10. : Telophase-II showing 6 groups of chromosomes (var. G_4). (0.03MMS)
11. : Anaphase-I showing unequal distribution (13:9) with 2 laggard (var. G_4). (0.04DES)
12. : PMC showing anaphase-I with 2-univalent and 2-bivalant as laggards (var. G_4). (0.05MMS)

Plate - III



An experimental field view (Faculty of Agricultural Sciences)

EXPLANATION OF FIGURES

Plate-IV

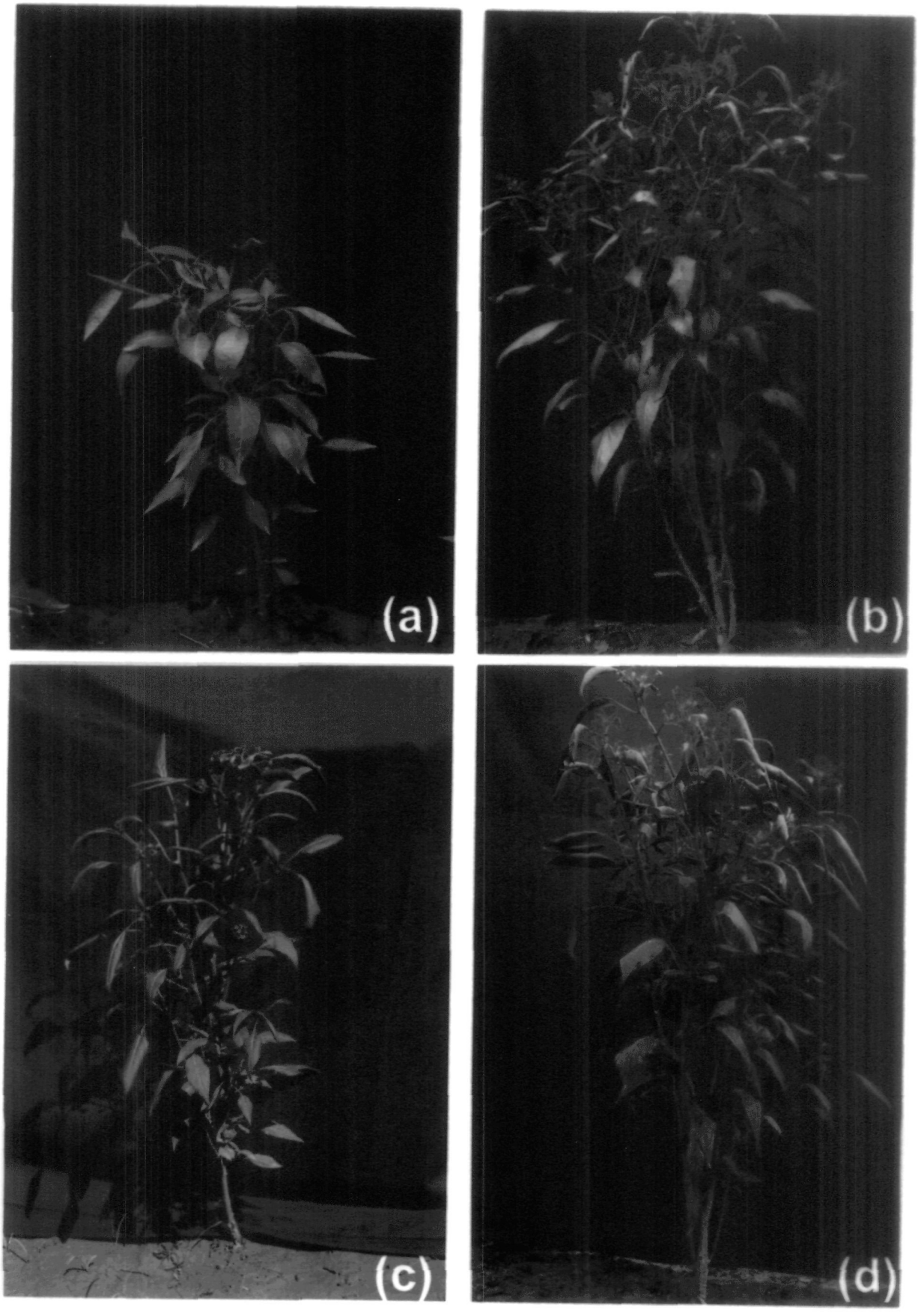
Fig. 1 : (a) Control (pusa jwala)

(b) Tall variant (0.03 MMS)

(c) Control (G_4)

(d) Tall variant (0.04DES)

PLATE-IV



EXPLANATION OF FIGURES

Plate-V

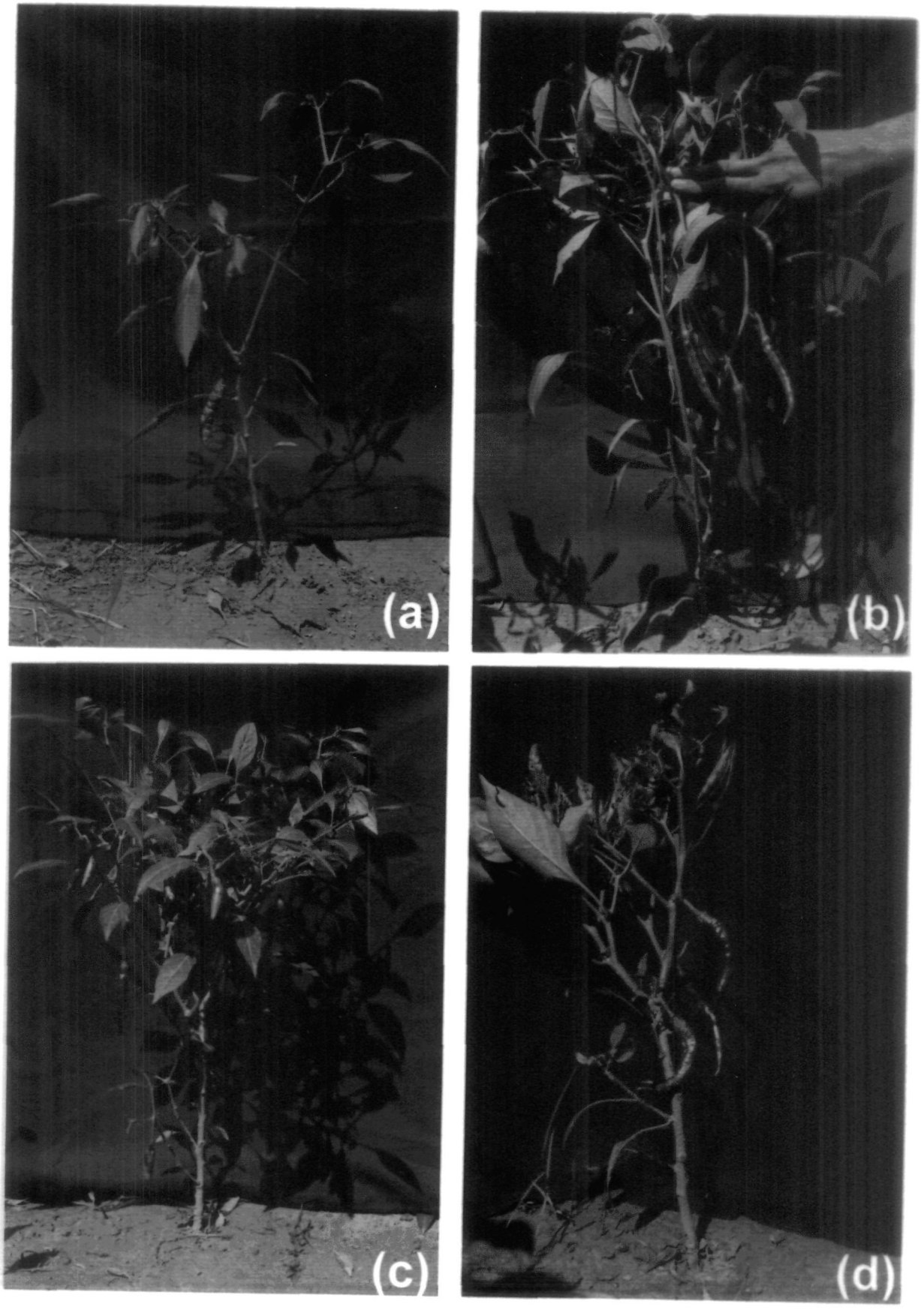
Fig. 2 : (a) Control (pusa jwala)

(b) Variant with long fruit (0.02DES)

(c) Control (G_4)

(d) Variant with long fruit (0.03MMS)

PLATE-V



EXPLANATION OF FIGURES

Plate-VI

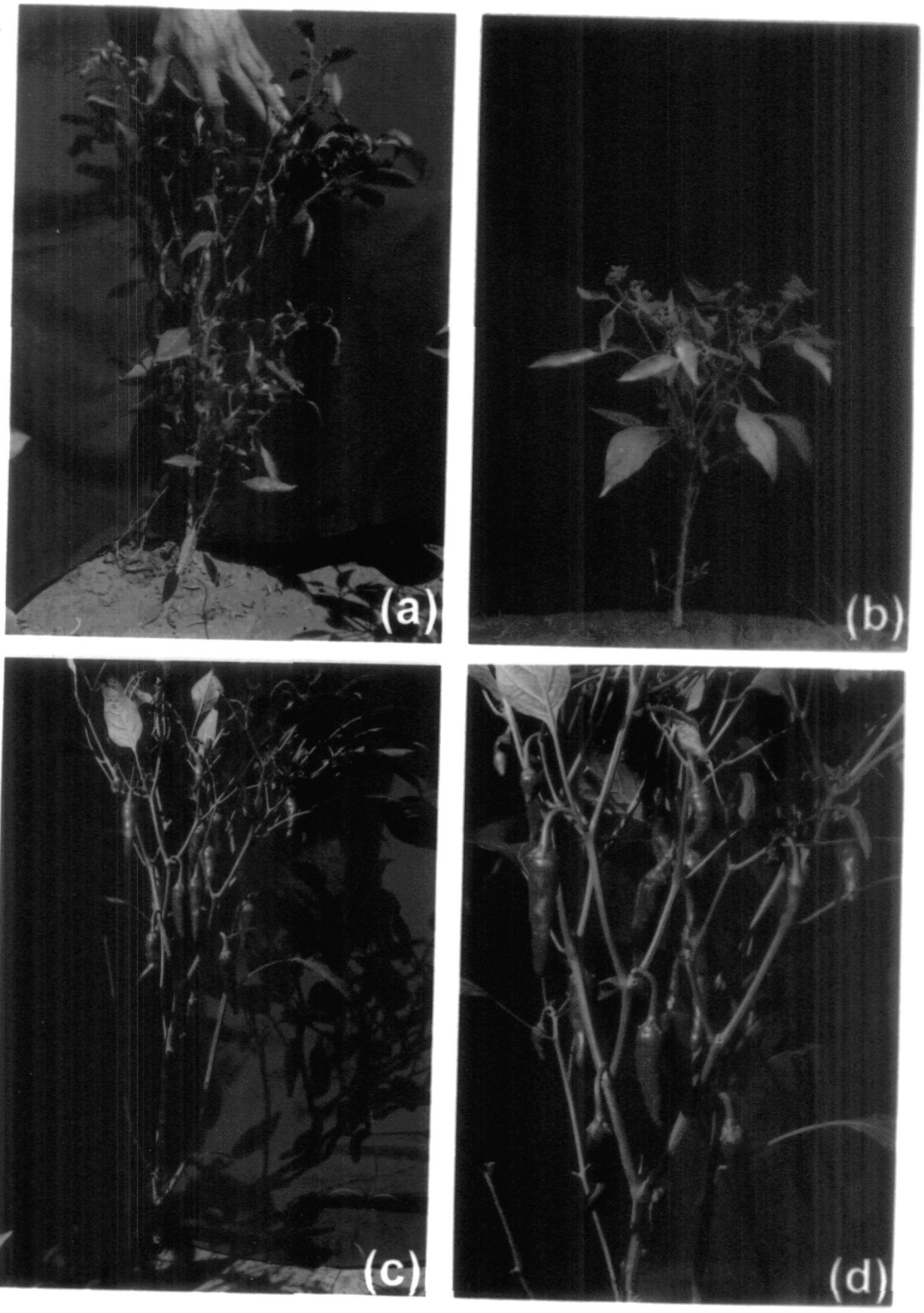
Fig. 3 : (a) Control (pusa jwala)

(b) Dwarf variant with short thick fruit (0.04MMS)

(c) Variant with large number of short thick fruits (0.02MMS)

(d) Closed view

PLATE-VI



EXPLANATION OF FIGURES

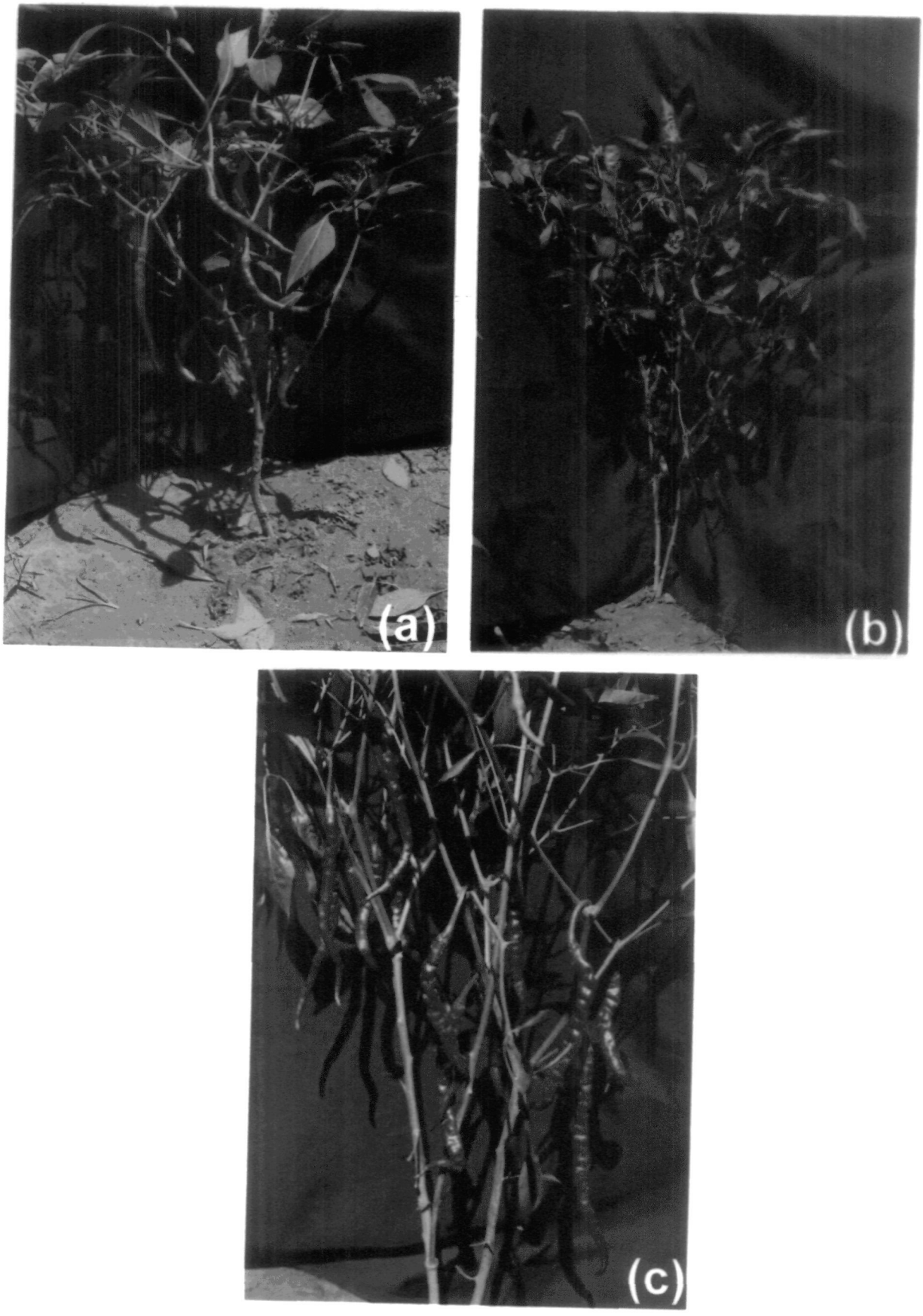
Plate-VII

Fig. 4 : (a) Control (pusa jwala)

(b) High yielding Variant (0.04MMS)

(c) Closed view

PLATE-VII



EXPLANATION OF FIGURES

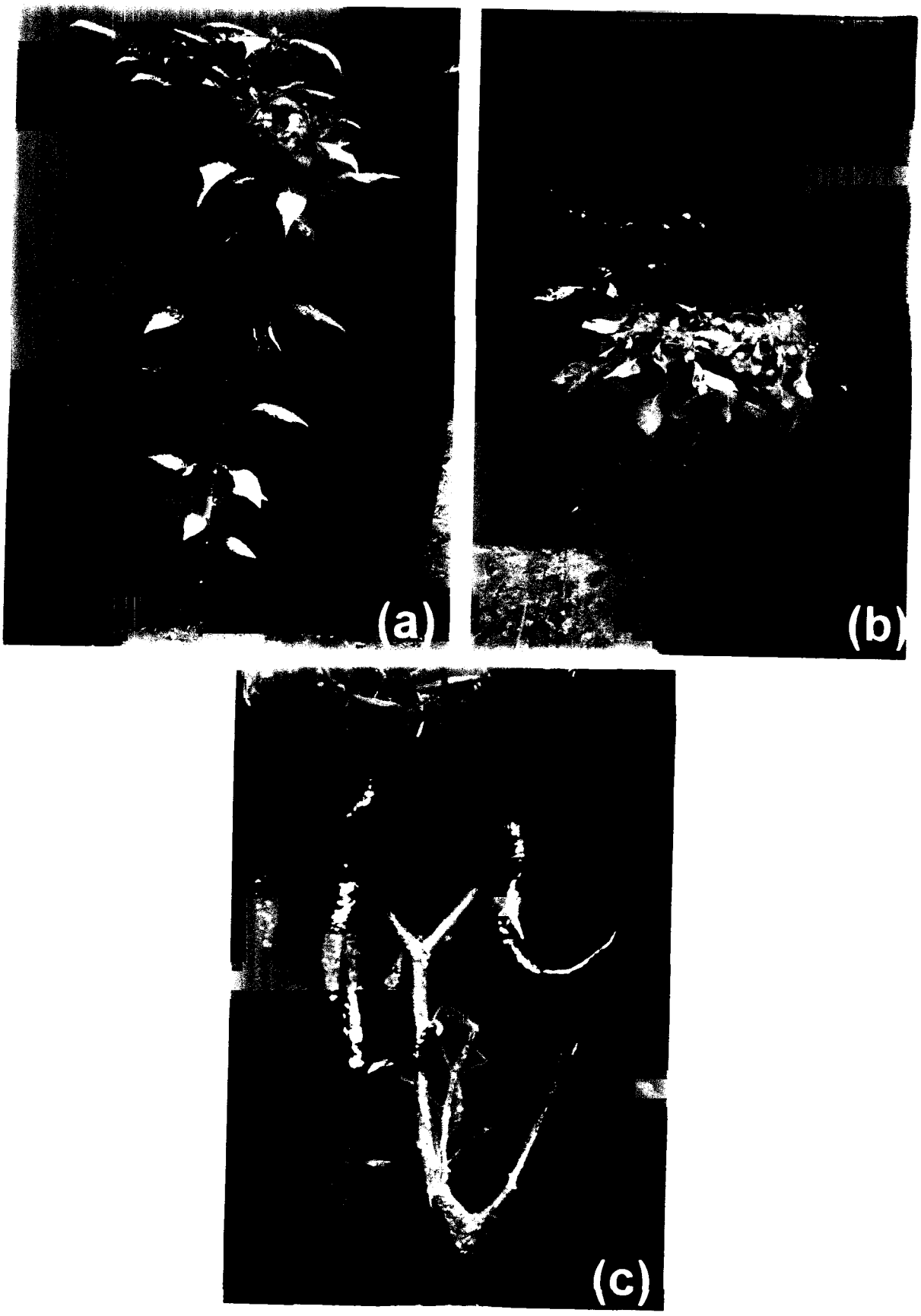
Plate-VIII

Fig. 5 : (a) Control (G_4)

(b) Dwarf high yielding Variant (0.03DES)

(c) Closed view

PLATE-VIII



EXPLANATION OF FIGURES

Plate-IX

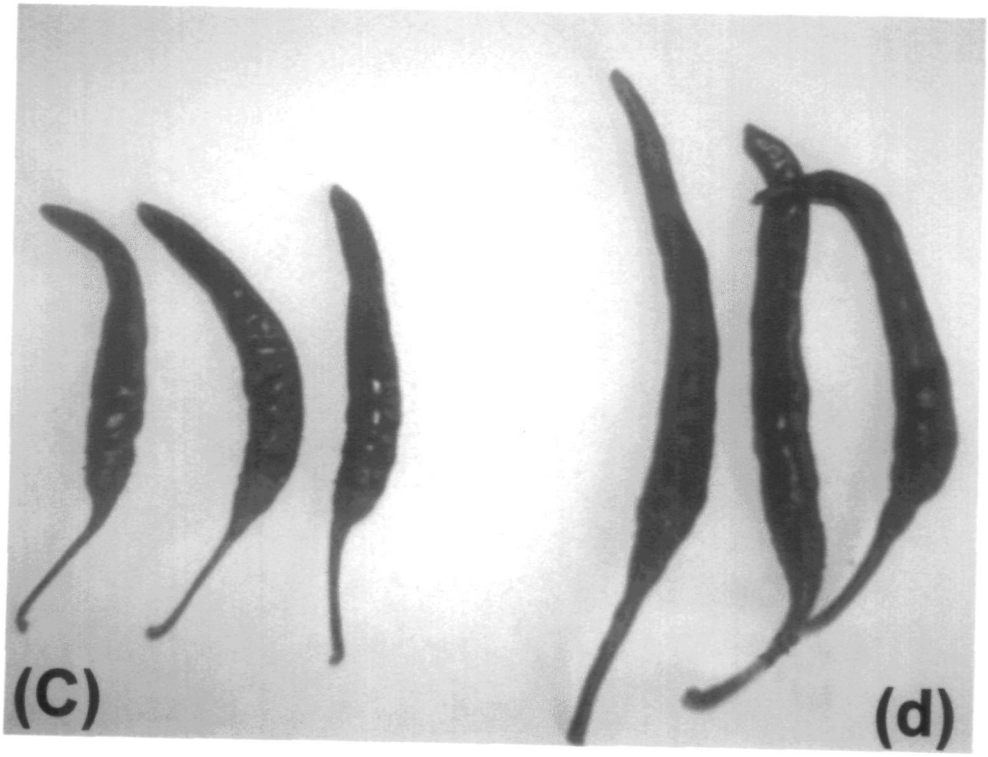
Fig. 6 : (a) Control (pusa jwala)

(b) Long fruit (0.2DES)

(c) Control (G_4)

(d) Long fruit (0.03MMS)

PLATE-IX



EXPLANATION OF FIGURES

Plate-X

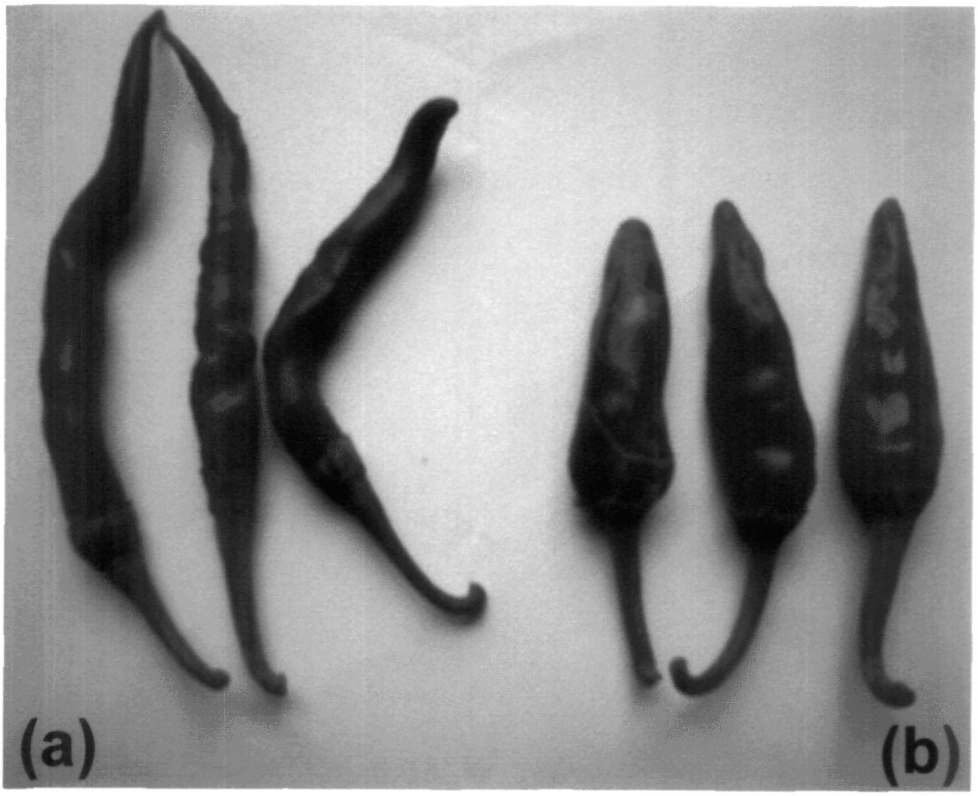
Fig. 7 : (a) Control (pusa jwala)

(b) Short thick fruit (0.04MMS)

(c) Control (G_4)

(d) Short thick fruit (0.02DES)

PLATE-X



Chapter-5
DISCUSSION

DISCUSSION

The present discussion is confined to the mutagenic effects of Methyl methane sulphonate (MMS) and Diethyl sulphate (DES) on cytomorphological characters of *Capsicum annuum* L. var. Pusa jwala and G_4 in M_1 generation. The results obtained during the course of present investigation have been discussed in this chapter.

5.1 Biological Damage:

(i) Seed Germination

In the present investigation seed germination, plant survival and pollen fertility decreased with the increase in concentration of the mutagens. These results are in conformation with *Wani et al* (2004) in lentil, *Potdukhe* and *Narkhede* (2002) in pigeon pea, Banu *et al.,* (2004) in cowpea and Kumar (2005) in *Coriandrum sativum*. The adverse effects of different mutagens on various biological parameters have also been reported by many workers *(Mehetre et al.*, 1990; *Kumar and Dubey,* 1998a; *Kumar and Mani* 1997; *Bhat et al.,* 2006). Most of these workers have observed a dose dependent reduction in the above mentioned biological parameters.

Kleinhofs (1978) in barley, reported delay in the initiation of metabolism following germination which in turn, resulted in a uniform delay in mitotic activity, seedling growth and ATP and DNA synthesis. Reduction in seed germination in mutagenic treatments

has been explained due to delay or inhibition in physiological and biological process necessary for seed germination which includes hormonal imbalance (*Chrispeeds and Varner, 1976),* defective enzyme production (*Kumar, 2005) and* inhibition of meiotic process (*Ananthaswamy et al., 1971).* The decrease in the percentage of seed germination after mutagenic treatment may be ascribed to the chromosomal aberrations, disturbances in DNA and auxin synthesis and to the impaired cell metabolism (*Kirtane and Dhumal, 2004).*

(ii) Plant Survival

Progressive decrease in the rate of survival of plants with an increase in the doses/concentration of physical and chemical mutagens has been reported by Jayabalan and Rao (1987a) in *Lycopersicon esculentum.* Decrease in survival may be attributed to the series of events at the cellular level which affect the vital macromolecules and bring about a physiological imbalance in the cells as a consequence of exposure to ionizing radiation and chemical mutagens. Physiological imbalance or different types of chromosomal aberrations or both may be the main cause for drastic decrease in survival (Rao, 1983).

(iii) Pollen Sterility

Varying degree of pollen sterility was induced in all mutagenic treatments in the present investigation. The magnitude of sterility increased with an increase in dose/concentration of mutagens. In some plants very high pollen sterility was recorded at the higher

doses of treatments. These results are in agreements with many workers who have also reported a dose dependent increase in pollen sterility following mutagenic treatments (*Kumar and Dubey 1994,1998a; Reddy and Annadurai, 1992*; *Dhamyanthi and Reddy 2000; Bhat et al., 2005a,b*). Mutagen induced pollen sterility was found to be chromosomal, genic, or physiological in nature (*Ramesh and Reddi, 2002).* The high sterility observed in the treated population may be attributed to the vast array of meiotic aberrations that were induced by physical and chemical mutagens leading to aberrant pollen mother cells and ultimately resulting in the inactivation of pollen grains (*Sinha and Godward, 1972a; Sharma et al., 2004; Bhat et al., 2005 a,b)*. Contrary to this, *Gaul et al., (1996)* in peas and barely reported that sterility was largely due to genetical changes. *Kumar and Mani* (1997) reported that the failure of homologous pairing during meiosis, could be the main cause of high pollen sterility. Kumar and Gupta (1978) attributed the high degree of sterility in *Vigna mungo* to asynpsis and/or desynepsis.

Sharma *et al.*, (2004) reported high pollen sterility in combination treatment (EMS+Gamma rays) than single treatment and suggested that induced sterility was mainly the result of chromosomal aberrations.

5.2 Cytological Analysis:

Cytological analysis with respect to either mitotic or meiotic behaviour is considered to be one of the dependable indices to estimate the potency of a mutagen. Therefore, investigations on disturbances in meiotic behaviour indicating mutational genetic load form an integral part of most of mutation studies. It also provides a considerable clue to assess the sensitivity of plants for different mutagens and to ascertain the most effective mutagen for a given crop to realize maximum results.

In the present investigation a vast array of meiotic aberrations were recorded in the plants raised from the seeds treated with different concentrations of MMS and DES. The different types of chromosomal aberrations viz. Univalents, multivalents, stickiness, precocious separation, chromatin bridges, laggards, and unequal separation (at anaphase I/II). Disturbed polarity, micronuclei, multinucleate condition. Cytomixis were observed in the present investigation. Similar results were also reported by many workers in different plants after treatments with physical and chemical mutagen, viz., *Anis and Wani (1997)* in *Trigonella foenum-graecum*; *Dhamyanthi and Reddi (2000)* in *Capsicum annuum*; *Singh (2003)* in *Vigna radiata*; *Rao and Laxmi (1980) and Ktiyar (1978)* in *Capsicum annuum* L., *Singh and Chaudhary (2005) in Chilli; Bhat et al. (2005 a)* in *Vicia faba, Kumar and Singh (2003)* in *Hordium* vulgare; *Kumar and Dubey (1998)* in *Lathyrus sativus*.

Most of these worker have obtained a dose dependent increase in the meiotic aberrations.

(i) Univalents

In the present study univalents were observed. Univalents have also been reported by Shah and Datta (2002) in *Nigella sativa*; *Kumar et al. (2003) in lense culinaris; Ganai et al. (2005) in chickpea; Bhat et al., (2006) in Vicia faba; Kumar and Rai (2006)* in *Glycine max* etc. Univalents may originate from an absence of crossing over at pachytene or from desyneptic mutant (Kumar and Rai, 2007). Chiasmata are responsible for the maintenance of bivalents, which permits normal chromosme segregation. It seems more likely that mutagenic treatments induced univalents formation through cryptic structural changes in some of the chromosome which restricted pairing and in this way reduced chiasma frequency (*Kallo 1972; Datta and Biswas 1986).*

Rao and Laxmi (1980) attributed univalents formation to the partial and complete lack of homologous chromosomes pairing. Further, the disturbances in the pairing mechanism was ascribed to the presence of chromosome breakage in the PMCs of plants raised from treated seeds. Some of the univalents disjuncted early and presumably this happened due to genic differences. Such chromosomal divergences in the form of precocious movement is pointed towards structural differentiation of homologous pair (*Anis and Wani, 1997*). *Mitra and Bhowmik (1996)* reported that non

pairing and early separation of chromosomes at meiosis and gene mutation may result in the formation of univalents. The chromosomes may fail to pair because of altiration in linearity of genes in them due to translocation and inversion induced by mutagenic treatments so that at early prophase homologous chromosome do not lie side by side. Mutagen induced structural changes in chromosomes and gene mutation might be responsible for the failure of pairing among homologous chromosomes and hence the presence of univalents (*Sharma et al.* 2004).

(ii) Multivalents

Different types of multivalent associations (tri, tetra, penta and chain of bivalents) observed in the present investigation have also been reported in various plants like *Trigonella foenum-graecum* (*Abbasi and Anis, 2002), Vicia faba* L. *(Bhat et al., 2006*). *Chickpea (Ganai et al., 2005).* Multivalents might have occurred due to chromosomal increases as an outcome of deletion resulting from fragmentation of chromosomes (*Singh and Gupta.* 2004) Multivalents formation can be attributed to irregular pairing and breakage followed by translocations and inversion. (*Zeerak. 1991*).

(iii) Stickiness

Stickiness of Chromosomes was one of the most common meiotic abnormalities observed in the present investigation. Chromosomes were found clumped into one, two or many groups due to stickiness at metaphase causing difficulty in normal

disjunction of chromosomes. These results are in agreements with those of *Mitra and Bhowmik (1996)* in *Nigella sativa*; *Kumar et al., (2003) in Barley; Bhat et al., (2006)* in *Vicia faba.* stickiness could be due to depolymerisation of nucleic acid caused by mutagenic treatments, (*Tarar and Dnyansagar, 1980*) or due to partial dissociation of the nueleoproteins and alteration in their pattern of organization (Sharma and Mukharjee, 1955). *Jayabalan and Rao (1987)* suggested that stickiness might be due to disturbances in the cytochemically balanced reactions. *Gaulden (1987)* attributed chemically induced stickiness to direct action of mutagen on the histone proteins leading to improper folding of DNA. Stickiness in chromosomes interfered in normal arrangement at metaphase, and further lead to their inability in separation and origin of thick sticky bridges.

(iv) Precocious Separation

Precocious movement of chromosomes at metaphase I was also observed in both the varieties treated with the mutagens. Similar results were also reported by different workers in different plants. (*Roy et al., (1971) in Cucumis sativus; Pagliarini (1990) in Apteria cardifolia; Defani-Scoarize et al., (1995a) in maize, Consolaro et al., (1996) in Centella asiatica* L. Precocious chromosome migration to the poles may have resulted from univalent chromosomes at the end of prophase-I or precocious chiasma terminalization at diakinesis or metaphase-I. Kumar and

Rai (2007). Un-orientation and scattering of chromosomes at metaphase as observed in the present study may either be due to the inhibition of spindle formation or destruction of spindle fibers.

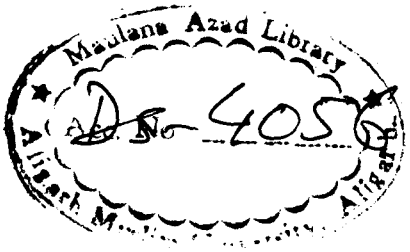
(v) Laggards

Laggard may be explained on the basis of abnormal spindle formation and chromosomal breakage. The laggards, observed during the present study might be due to delayed terminalization, stickiness of chromosomal ends or because of chromosome movement (*Jayabalan and Rao, 1987; Soheir et al., 1989; Permjit and Grover 1985).* According to *Bhattacharjee (1953)* acentric fragment or laggards may results in the formation of micronuclei at Telophase-II. Laggards at anaphase have been observed by *Ganai et al., (2005)* in chickpea, *Kumar and Rai (2007)* in maize, Singh and Chaudhary (2005) in chilli, *Abbasi and Anis (2002),* in fenugreek, laggards may arise by breakage or faulty spindle resulting into imblanced daughter nuclei and micronuclei (*Singh and Chaudhary, 2005).* Presence of laggards may be attributed to the inability of multivalents to separate properly (*Ganai et al., 2005).*

(vi) Bridge

Bridges with or without fragment were also observed in the present study. *Saylor and Smith (1996)* suggested that the formation of chromatin bridges might be due to the failure of chiasmata in a bivalent to terminalise and the chromosomes get stretched between the poles. *Bhattacharjee (1953)* attributed

bridge formation to interlocking of bivalent chromosomes. The occurrence of breaks of the same locus and their lateral fusion leads to the formation of dicentric chromosome which is pulled equally to both the polls forming a bridge (*Anis et al.,* 1998). *Sinha and Godward (1972)* attributed bridge formation to paracentric inversion. Bridges often breaks as the dyads move further apart in the late anaphase, sometimes leaving an acentric fragments in the cytoplasm. The fragments are seen during meosis-II as micronuclei which do not condense alongwith remaining chromosomes. The presence of chromosome bridges without fragments may be due to restitution or the fragments getting entangled or attached with normal chromatids of chromosomes *(Tarar and Dnyansagar, 1980).* Moreover, PMCs with a single bridge without acentric fragment at anaphase I was formed by two sister chromatids of a broken chromosome which has undergone fusion during interphase at the time of duplication. In the present study, bridge formation may be attributed to the general stickiness of chromosomes at metaphase stage or breakage and reunion of chromosomes. The present findings are in agreement with the ealier results of *Jayabalan and Rao (1987) in tomato, Kumar and Rai in (2007) in maize, Singh and Chaudhary (2005)* in chilli etc. *Gaul (1964)* attributed anaphasic bridge in barley to the fusion between two centromere bearing chromosome fragments.



(vii) Unequal Separation

Unequal separation of chromosomes as observed in the present study, may be due to failure of chromosome to reach the poles. *Sinha and Godward (1972)* attributed the unequal distribution to the occurrence of multivalents and failure of chromosomes to segregate equally. This may be due to spindle disfunciton caused by mutagens (*Nerker, 1977; Singh et al., 1989; Grover and Virke 1986).* According to *Kumar* and *Singh (2003)* random movements of univalents to any one of the poles leads to the unequal separation of chromosomes. Stickiness of the chromosomes may also result in the unequal distribution of chromocomes in the daughter nuclei (*Anis and Wani, 1997).* It was also reported by *Sharma and Kumar (2004)* in *Cicer arietinum* and *Ganai et al., (2005)* in chickpea, *Abbasi and Anis (2002)* in fenugreek and Kumar and Rai (2007) in Maize.

Abnormalities such as lagging chromosomes and unequal separation of chromosomes specially the last one would lead to the production of aneuploid gametes. Such plants (aneuploids) are of immense importance in fundamental as well as applied research in crop improvement.

(viii) Micronuclei

Micronuclei as observed in the present study at telephase II generally arose from fragments and lagging chromosomes which failed to reach the poles and get included in the daughter nuclei

(Kumar and Dubey 1998b). Laxmi et al., (1975) and *Bhattacharjee (1953)* suggested that irregular distribution of acentric fragments or laggards results in the formation of micronuclei at telephase resulting in variation in number and size of pollen grains obtained from the pollen mother cell. Micronuclei lead to the loss of genetic material. Their presence, therefore, suggested that the resultant product of meiotic division is deficient in one or the other chromosome. This usually leads to the formation of the sterile pollen grains. The presence of micronuclei at telophase-II is also reported by *Kumar and Rai (2007) in maize, Ganai et al.,* (2005) in Chickpea.

(ix) Disturbed polarity

Disturbed polarity at anaphase and telophase stages could be due to spindle disturbances. Disturbed polarity was also reported by Bhat *et al.,* (2006) in *Vicia faba* L., *Kumar and Rai* (2007) in maize, *Ganai et al., (2005)* in chickpea and *Abbasi and Anis (2002) in fenugreek.*

(x) Cytomixis

In the present investigation, very low frequency of cytomixis at various stages of meiosis was noticed. It has also been reported more commonly during microsporogenesis by several workers *(Sudan and Wafai, 1987; Bahal and Tyagi, 1988; Kaul 1990; Kumar and Sharma, 2002; Bhat et al., 2006; Kumar and Rai, 2007).*

Cytomixis referes to the migration of chromatin/Chromosome from one cell (donar cell) to the cytoplasm of other cell (recipient cell) either through cytoplasmic channels or through direct fusion at different stages of meiosis. Gates (1911) coined the term cytomixis for transition of structureless chromatin drops while Gottschalk (1970) designated it as "Chromosome and Nucleus migration," In the present study cytomixis observed, has been considered as transfere of nuclear material from donor to recipient cells which may involve cytoplasm as well. The causes of cytomixis are rather ambiguous. Suggestion regarding the possible cause of cytomixis include physiological, pathological factor (Bobak and Herick 1978); effect of fixation (Takats, 1959); Nutritional deficiency (Milajajev, 1967); as well as genetically controlled behaviour (Brown and Bertke 1974). Cytomixis was responsible for sterility because of the fact that firstly, the number of pollen would be considerably reduced due to degeneration of cell with no or very little genetic material and secondly, most of the cell completing all the meiotic stages, would be genetically imbalanced because of less or more number of chromosomes than the normal ones.

The ultimate result of these aberrations after normal or abnormal cytokinesis was the formation of abnormal microspores leading to pollen sterility (*Ramanna, 1974)* which was directly correlated to doses administered and the magnitude of clastogenic changes induced by mutagens in both the varieties.

(xi) Chiasma frequency

Chiasma frequency characterises the pairing of homologous chromosomes at meosis and controls the degree of recombinations besides influencing the fertility. It provides the organisms with a mechanisms for adaptation to new habitat (Sun and Rees 1964). Extensive studies on chiasma frequency have been made by many workers. Mutagenic agents are known to bring about changes in chiasma frequency. Sinha and Roy (1976) in *Phaseolus,* Sadanandam and Subhash (1984), and Kumar and Rao (1985) in *C. annuum.*, Reddy and Annadurai (1992) in lentil reported a reduction in chiasma frequency.

In the present investigation, the chiasma frequency per cell as well as per bivalent decreased in all mutagenic treatments in both the varieties of *Capsicum annuum* L. The reduction in chiasma frequency may be attributed to the nature and potency of the mutagens and also to the underlying factors such as complex structural changes or due to change in the nature of the gene responsible for chiasma formation (Anees and Sharma 1997). Rees (1955) showed that crossing over and chiasma formation are under genetic control where as Gottschalk and Klein (1976) reported that chiasma formation and its frequency is controlled by large number of genes. The low chiasma frequency in the treated population may also be attributed to the failure of complete pairing. Reduction in chiasma frequency after mutagenic treatments have also been

reported by many workers (Singh *et al.*, 1977; Sadananadam and Subhash, 1984; Laxmi *et al.*, 1998; Jayabalan and Rao, 1987; Bhat *et al.*, 2006). Sadanandam and Subhash (1984) attributed the reduction in chiasma frequency to the nature and potency of mutagens, structural changes or the change in the nature of the gene responsible for chiasma formation. It might also be attributed to the failure of complete pairing.

Jain and Bask (1965) reported that mutagenic treatemnets induced univalent formation through cryptic structural changes in some of the chromosomes which restricted the pairing and in this way reduced the chiasma frequency.

5.3 Quantitative Characters

The useful variability is a pre-requisite for crop improvement programme. Therefore, the first step in any breeding programme would be the search for such variability. Mutagenesis has provided a handy tool to enhance the natural mutational rate and thereby enlarging the genetic variability and increasing the scope for obtaining the desired selections. The practical utility of induced mutations for polygenic traits is well established, since most of the economic characters in crop species are quantitatively inherited. Particularly induction of micromutations in polygenic system, controlling the quantitative characters are important for crop improvement. In the recent years the role of mutation breeding in increasing the variability for quantitative characters has been

proved beyond doubt (*Mehetre et al., 1990; Srivastava and Singh, 1993; Ignacimuthu and Babu, 1993; Waghmare and Mehra, 2000).*

The mutagenic effect of various concentrations of MMS and DES was studied on days to flowering, days to maturity, plant height (cm), number of fruit per plant, length of fruit, girth of fruit, and total yield per plant. Assessment of range, mean and coefficient of variation (C.V.%) in control and treated populations indicated that mutagenic treatments have induced wider magnitude of variability in M_1 generation for all the quantitative characters. The mutagenic effect was clearly seen at different concentrations of all the mutagens leading to both increase and decrease in the mean values of these quantitative traits. The positive shift of mean values was more pronounced at the lower dose treatments, whereas, negative shift was observed at higher doses except for days for flowering and days to maturity where reverse was true.

In the mutagen treated populations the pooled mean values of different mutagens for different quantitative character remained mostly unchanged or caused a very little change because of shift in both the directions despite significant increase in variance indicated that the mean values of these quantitative traits of different concentrations were not by and large altered at greater extent in both the varieties. Shift in mean values in both positive and negative directions after mutagenic treatments has been reported by earlier workers (*Abdalla and Hussein, 1977; Sinha and Joshi,*

1986; Mehetre et al., 1990; Waghmare and Mehra 2000; Singh et al., 2000b). Most of the quantitative characters have a complex genetic determination involving large number of genes interacting with one another. Consequently variation in both the directions is expected. Since not very much appreciable changes were noticed in the pooled mean values for various quantitative characters in the present investigation, it is assumed to be due to the fact that in the present study macro-mutational variants were excluded from the assessment of mean in M_1 generation and data were recorded from the normal looking plants only. Reduction in the mean values of above quantitative traits and also delayed flowering and maturity were common feature of mutagenic treatments at higher dose level noticed in the present investigation. This has also been reported by various earlier workers (*Nerker 1970; Anis et al., 1999; Anis and Wani, 1997; Kumar and Dubey, 1994, 1998a; Bhat et al., 2006b).*

In the present investigation the mean days to flowering, and days to maturity decreased at the lower concentrations and increased significantly at the higher concentrations of the mutagens. Reduction in flowering and maturity time after mutagenic treatments have also been reported in urdbean (*Singh et al., 2000b)*, in grass pea (Waghmare and Mehara 2000). Variation in flowering and maturity period is generally considered to have parallel relation, however, antiparallel relation has also been reported (*Porsche, 1963).*

Plant height decreased considerably in the treated populations. Similar findings in this respect have been reported by *Banu et al.* (2004) *Ramesh and Reddi (2002).* Reduction in the plant height may be due to the chromosomal damage and/or inhibition of cell division (*Thoday, 1951; Sparrow et al. 1961)* whereas, *Goud and Nayar (1968)* and *Tarar and Dnyansagar (1980)* demonstrated that growth depression might be due to inhibition of auxin synthesis. *Bansal et al. (1967)* ascribed reduction in height to the shortening of internodes. Therefore, reduction in the growth of plant might have occurred due to inhibitory effect of mutagens on growth regulating substances responsible for cell division and cell elongation.

In the present investigation the mean values of quantitative characters like number of fruit per plant, length and girth of fruit), and total yield per plant showed significant increase at the lower concentrations of the mutagens, while higher concentrations showed inhibitory effect. The inhibitory effect at high concentrations leading to decreased yield was due to prohibitory action of enzymes concerned with the initial growth processes and changes in the enzyme activity (*Blixt et al. 1963).* These characters play an important role in boosting the yield of a plant to a considerable extent. Relation of yield with other quantitative characters has also been studied earlier by *Gottschalk and Kaul 1975; Khan et al., 1999).* The possible cause of increasd value of these yield

contributing characters in the present study may be due to some useful gene mutations and chromosomal aberrations.

In the present study although no linear relationship was observed between the concentrations and mean values for all polygenic characters but varietal differences in terms of mutagenic response was clearly evident. Such varietal differences have also been reported earlier by most of the workers (*Sharma and Sharma, 1981a; Kharkwal, 1988a and Khan, 1999).* The occurrence of mutation with equal frequency towards positive and negative direction may be considered as an important reason to justify the tendency of positive and negative shifts in the mean values for various quantitative characters in the treated populations. As stated earlier, a considerable range of polygenic variability measured in terms of coefficient of variations was induced by all mutagenic treatment in both the varieties. However, the amount of induced variability varied not only among different treatments but also from character to character.

In brief, the present finding have revealed that lower and moderate doses/concentrations of chemical mutagens proved to be efficient in increasing the genetic variability for yield oriented selection in *Capsicum annuum* been and the isolated variants possessed desirable characters associated with higher yield may be evaluated in future generations for promising traits. Thus the

genetic variability induced by mutagenesis may effectively be exploited for the improvement of broad *Capsicum annuum L.*

(SUMMARY AND CONCLUSION)

The present investigation deals with the studies of the effect of chemical mutagens i.e., MMS and DES on cytomorphological parameters of *Capsicum Annuum* L. var. pusa jwala and G_4 in M_1 generation. An Attempt has been made to explore the possibilities of inducing alterations in the geneotype to enhance the genetic variability and to increase the yield potential of *Capsicum Annuum* L. through the use of above said mutagens. The findings are summarized below.

(1)All biological parameters viz., seed germination, plant survival and pollen fertility were decreased with an increase in Mutagenic concentration in both the varieties.

(2)Lower doses of mutagen exhibit stimulatory effect on various economic characters viz., plant height, days to flowering, days to maturity, no. of fruits per plant, fruit length, fruit girth. and total yield per plant.

(3)Different types of meiotic abnormalities were observed in treated populations in different frequencies. The number of abnormalities showed dose dependent increase in both the varieties treated with mutagens.

(4)The meiotic abnormalities observed in different concentrations of mutagens were univalents, multivalents, stickiness, stray bivalent,

precocious separation, cytomixis, disturbed polarity and micronuclei etc.

(5)Chromosomal aberrations found in *Capsicum annuum* L. var. pusa jwala and G_4 represents its genetic sensitivity which provides clue for its genetic improvement through mutation breeding.

(6)Mathematical and statistical analysis of data recorded in M_1 generation particularly on biological and economic characters have been carried out to stablish the statistical relationship between concentration of MMS and DES and different parameters of *Capsicum annuum* L.

(7)There is an increase as well as decrease has been found in the mean values showing a positive as well as negative shift in the different biological and economic characters, whereas the coefficient of variability was considerably high among the treated populations as compared to control.

(8)The results obtained in the present work has been discussed in the light of earlier investigations carried out on different groups of plants.

Bibliography

BIBLIOGRAPHY

Abbasi, N. and Anis, M. (2002). Clastogenic effect of chemical mutagens in *Trigonella foenum-graecum* L. *J. Cytol. Genet.*, **3 :** 109-114.

Abdalla, M.M.F. and Hussein, H.A.S. (1977). Effects of single and combined treatments of gamma rays and EMS on M_2 quantitative variation in *Vicia faba* L.Z. *Pflanzenzuecht*, **78**: 57-67.

Alkantara, T.P. Basland, P.W. and Smith, D.W. (1996). Ethylmethane sulphonate-induced seed Mulagenesis of *Capsicum annuum* L. The Journal of heredity, **87**: 239-241.

Altenberg, E. (1928). The limit of radiation frequency effective in Producing mutations. Amer. nat., **62**: 540-545.

Ananthaswamy, H.N., Vakil, U.K. and Srinivasan, A. (1971). Biochemical and physiological changes in gamma irradiated wheat during germination *Rad. Bot.*, **11**: 1-12.

Anis, M. and Sharma, N. (1997). Induced variations in chiasma frequency in *Capsicum annuum* L. in response to chemical mutagens. Thai. J. Agric. Sci., **30**: 43-52.

Anis, M. and Wani, A.A (1997). Caffeine induced morpho-cytological variability in fenugreek, *Trigonella foenum-graecum* L. *Cytologia,* **62**: 343-349.

Anis, M., Abbasi, N. and Sharma, N. (2000). Cytological effects of mutagens in *Capsicum annuum* L. J. Cytol. Genet., **1**: 17-21.

Anis, M., Sharma, N. and Abbasi, N. (1998). Colchicine induced autoteraploidy in *Capsicum frutescens* var. Suryamukhi, J. Cytol. Genet., **33**: 183-187.

Anis, M., Shiran B. and Wani, A. (1998). Genotoxic effect of aldrin and malatheon on the root meristem of *Vicia faba* L. *J. Cytol. Genet.*, **33**: 35-42.

Asha, M.A. and Nayar, N.K. (1986). Influence of genic status in relation to gamma rays and EMS induced pollen sterility in chillies (*Capsicum annuum* L.) Indian. J. Bot., **9**: 89-91.

Bahl, S.R. and Tyagi, B.R. (1988). Cytomixis in pollen mother cells of *Papaver dubeum* L. *Cytologia,* **53**: 771-775.

Bansal, S.K., Singh, H.C.D. and Swaminathan, M.S. (1967). Pathway of height reduction in induced dwarf mutation in barley. *Z. pflanzenzuchtg,* **57**: 317-324.

Banu, M.R., Ashok, S. and Kalamani, M. (2004). Effect of mutagenic treatments in M_1 generation of cowpea (*Vigna unguiculata* (L.) Walp.). *Int. J. Mendel,* **21**: 63-64.

Bhat, T.A., Khan, A.H. and Parveen, S. (2005a). comparative analysis of meiotic abnormalities induced by gamma rays, EMS and MMS in *Vicia faba* L. *J. Indian Bot. Soc.,* **84**: 45-48.

Bhat, T.A., Khan, A.H. and Parveen, S. (2006a). Effect of EMS on certain Cytomorphological parameters in two varieties in *Vicia faba* L. *Ad. Plant Sci.,* **19**: 227-232.

Bhat, T.A., Parveen, S. and Khan, A.H. (2005b). Clastogenic effect of EMS and MMS in *Vicia faba* L. *J. Cytol. Genet.,* **6**: 117-122.

Bhattacharjee, S.K. (1953). Cytogenetics of *Lens esculenta* Monesh. *Caryologia*, **5**: 159-166.

Blixt, S., Eherenberg, L. and Gelino (1963). Studies on induced mutation in peas. VII. Mutation spectrum and mutation rate of different mutgenic agents. *Agri. Hot. Genet.,* **21**: 178-216.

Bobak, M. Herich R. (1978) Cytomixis as a manifestation of pathological changes after the application of trifluraline *The Nucleus,* **21:** 22-26.

Brock, R.D. (1970). Mutations in quantitatively inherited traits induced by neutron radiation. Rad. Bot., **10**: 209-223.

Brown, W.V., Bertke E.M. (1974) In: Text book of cytology, 2nd ed, Mosby CV, Saint Louis.

Chaturvedi, S.N. and Singh V.P. (1980). Gamma radiation induced quantitative variation in mungbean. *J. Cytol. Genet.*, **15**: 64-67.

Chennaveeraiah, M.A. and Habib, A.F. (1973). Cytomorphological studies in spontaneous triploid of *Capsicum annuum* L. Cytologia, **38**: 677-685.

Chrispeeds, M.J. and Varner, J.E. (1976). Gibberellic acid induced synthesis and release of L-amylase and ribonuclease by isolated barely aleurone layers. *Plant Physiol.* **42**: 396-406.

Consolara, M.E.I., Pagliarini, M.S. and Chaves, L.J. (1996). Meiotic behaviour of pally feruling and seed production in Brazilian population of *Centella asiatica* (L.) Urban (Umbelliferea). Cytologia, **61**: 375-381.

Datta, A.K. and Biswas, A.K. (1986). Cytogenetic studies in two induced leaf mutant lines of *Nigella sativa* L. *Cytologia,* **51**: 309-317.

Defani-Scoarize, M.A., Pagliarini, M.S. and Aguiar, C.G. (1995a). Causes of partial male sterility in an inbred maize line. Cytologia, **60**: 311-318.

Dhamyanthi, K.P.M. and Reddy, V.R.K. (2000). Cytogenetic effect of gamma rays and ethyl methane sulphonate in chilli pepper *(Capsicum annuum* L.*). Cytologia,* **65**: 129-133.

Dixit, P. and Dubey, D.K. (1986). Alkylating agents induced chromosomal aberrations in lentil (*Lens culinaris* Med. Var. T. 36) Cytologia, **51**: 325-331.

Edwin, R. and Reddy, V.R.K. (1993). Effects of gamma rays and EMS an certain biological parameters in Triticale, J. Indian Bot. Soc. **72**: 97-101.

Ganai, F.A., Khan, A.H., Bhat, T.A., Parveen, S. and Wani, N.A. (2005), Cytogenetic effects of methylmethane sulphoante (MMS) on two varieties of chickpea (*Cicer arietinum* L.), *J. Cytol. Genet.,* **6**: 97-102.

Gates, R.R. (1911) Pollen formation in Oenothera gigas Ann Bot., **25:** 909-940.

Gaul, H. (1964). Mutations in plant Breeding. *Rad. Bot.,* **4**:155-232.

Gaul, H., Bender, K., Ulonska, E. and Sato, M. (1966). EMS induced genetic variability in barley. The problem of EMS induced sterility and method to increase the efficiency. In Proc. Intl. Panel on Mutations in Plant Breeding. 63-84.

Gaulden, M.E. (1987). Hypothesis: Some mutagens directly alter specific chromosomal protein to produce chromosome stickiness. *Mutagenesis,* **2**: 357-365.

Goodspeed, T.M. (1929). The effect of x-ray and radium on species of genus Nicotiana. J. Hered., **20**: 245-255.

Gottschalk, W. and Kaul, M.L.H. (1975). Gene ecological investigations in Pisum Mutants. I. The influence of climatic factors upon quantitative and qualitative characters. *Z. pflanzenzuchtg,* **75**: 182-191.

Gottschalk, W. (1970) Chromosome and nucleus migration during microsporogenesis of *Pisum sativum*. The Nucleus **13:** 1-9.

Gottschlak, W. and Kleion , H.D. (1976) The influence of mutated genes on sporogenesis,. A survey on the genetic control of meiosis in pisum sativum. Theor. Appl. Genet., **48:** 23-24.

Goud, J.V and Nayar, M.D. (1968). Effect of irradiation on seedlings of methi. *Mysore J. Agric. Sci.*, **11**: 53-55.

Goud, J.V. (1965). Studies on the frequency and spectrum of visible and micromutations induced by some physical and chemical mutagens in varieties of bread wheat. Ph.D. thesis, IARI, New Delhi.

Grover, I.S. and Virk, G.S. (1986). A comparative studies of gamma rays and some chemical mutagens on the induction of chromosomal aberrations in mungbeans (*Vigna radiata* L.). *Acta Botanica Indica,* **14**: 170-180.

Harini, I. Lakshmi, N; and Prakash, N.S. (1990). A new report of a chromosomal chimaeral in *Capsicum annuum* L. Cytologia, **55**: 649-654.

IAEA. (1970). Manual of mutation breeding. International Atomic Energy Agency, Vienna.

IAEA. (2000). Plant breeding and genetic newsletter FAO/IAEA. No.5, June, 2000.

Ignacimuthu, S. and Babu, C.R. (1993). Induced quantitative variation in wild and cultivated urd and mungbean. *J. Nuclear Agri. Biol.*, **22**: 133-137.

Indira, C. and Abraham, S. (1977). Morphological and cytological studies on a radiation induced polyloidy in *Capsicum annuum* L. Cytologia, **42**: 271-375.

Jabee, F. and Ansari M.Y.K. (2005). Mutagenic effectiveness and efficiency of Hydrazine sulphate (HS) in inducing cytomorphological mutations in *Cicer areitinum* L. var. K. 850. J. Cytol. Genet. L. **6**: 161-

Jain, S.C. and Agarwal, M. (1993). Changes in morphological characters in *Trigonella foenum-graecum* L. due to chemical mutagens. J. Indian Bot. Soc., **72**: 277-280.

Jayabalan, N. and Rao, G.R. (1987). Gamma radiation induced cytological abnormalities in *Lycopersicon esculentum* Mill. Var. Pusa ruby. *Cytologia,* **52**: 1-4.

Jaybalan, N. and Rao, G.R. (1987a). Effect of physical and chemical mutagens on seed germination and survival of seedling in *Lycopersicon esculentum* Mill. *J. Indian Bot. Soc.* **10**: 133-137

Kallo, C.S. (1972). Chromosomal alterations in mitotic and meiotic system as influenced by gamma rays in *Pisum. Cytologia,* **37**: 643-651.

Katiyar, R.B. (1978). Radiocytogenetical studies in *Capsium* I. Meiotic anomalies. *Cytologia,* **43**: 415-421.

Kaul, K.K. (1990) Cytomixis in Pollen mother cells of Alopecurus arundinaceous poir. Cytologia **55:** 169-173.

Khan, I.A. (1984). Mutations inducd by gamma irradiation, EMS and HZ in mungbean (*Phaseolus.aureus* Roxb.). Bot. Bull. Acad. Sin (Teipei), **25**: 103-110.

Khan, I.A. and Hashim, M. (1978). Radiation induced variability in quantitative traits of mungbean *(Phaseolus aureus* Roxb.). J. Cytol. Genet., **13**: 12-15.

Khan, M.N. (1999). Mutagenic effectiveness and efficiency of EMS, gamma rays and their combinations in balckgram (*Vigna mungo* L. Hpper). *Ad. Plant Sci.,* **12**: 203-205.

Khan, S., Siddiqui, B.A. and Rehman, M. (1999). Mutation genetic studies in mugbean. III. Screening of high yielding mutants. *J. Cytol. Genet.,* **34**: 75-78.

Kharkwal, M.C. (1988a). Induced mutations in chickpea (*Cicer arietinum* L.) I. comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. *Indian J. Genet.,* **58**: 159-167.

Kirtane, S. and Dhumal, K.N. (2004). Studies on induced mutations in onion: Biological and cytological effects of mutagens in M_2 generation. *Int. J. Mendel,* **21**: 11-13.

Kleinhofs, A., Owais, W.M. and Nilan, R.A. (1978). Azide. *Mut. Res.*, **55**: 165-195.

Kumar, A. (2005). Role of ethyl methane sulphonate on the germination of *Coriandrum sativum* L. *Int. J. Mendel,* **22**: 29.

Kumar, G. and Rai, P.K (2005). EMS indcued genetic variability in soybean (*Glycine max*). The nucleus., **48**: 46-51.

Kumar, G. and Rai, P.K. (2006), Induced desynapitc male sterile lines in soybean. *Cytologia,* **71**: 337-343.

Kumar, G. and Rai, P.K. (2007). EMS induced karyomorphological variations in maize (*Zea mays* L.) Inbreds. *Turk. J. Biol.,* **31**: 187-195.

Kumar, G. and Sharma, V. (2002). Induced cytomixis in chickpea (*Cicer arietinum* L.). *The Nucleus.,* **45**: 24-26.

Kumar, G. and Singh, V. (2003a). Comparative analysis of meiotic abnormalities induced by gamma rays and EMS in barley. *J. Indian Bot. Soc.,* **82**: 19-22.

Kumar, G. and Gupta P. (2009) Induced karyomorphological variations in three phenodeviants of *Capsicum annuum* L. Turkjbiol., **33:** 123-128.

Kumar, G., Kesarwani, S. and Sharma, V. (2003). Clastogenic effect of individual and combined treatments of gamma rays and EMS in *Lens culinaris. J. Cytol. Genet.,* **4**: 149-154.

Kumar, O.A. and Raja Rao K.G. (2006). Cytogenetics of spontaneous fascinated stem mutant of chilli peper (*Capsicum annuum* L.). Cytologia, **71**: 321-324

Kumar, O.A. and Raja Rao, K.G. (1985). Desynepsis in *Capsicum frutescence* L. Cytologia, **50**: 69-73.

Kumar, O.A. and Raja Rao, K.G. (2003). Cytomorphological studies in gamma rays induced autotriploids of *Capsicum annuum* L. Cytologia, **68**: 45-50.

Kumar, R. and Mani, S.G. (1997). Chemical mutagenesis in Manhar variety of rice (*Oryza satva* L.). *Indian J. Genet.*, **44**: 80-83.

Kumar, S. and Dubey, D.K. (1994). Effect of chemical mutagens on germination, growth, fertility and yield in yellow sarson (*Brssica compestris* L.) var. Dira-31.3. *Indian Bot. Rept.*, **16**: 19-24.

Kumar, S. and Dubey, D.K. (1998a). Effect of physical and chemical mutagens and their interactions on germination, growth, fertility and yield in two cultivars of Khesari. *J. Phytol. Res.*, **11**: 147-151.

Kumar, S. and Dubey, D.K. (1998b). Effect of separate and simultaneous application of gamma rays and EMS on germination, growth, fertility and yield in cultivars Nirmal and LSD-3 or Khesari (*Lathyrus sativus* L.). *J. Phytol. Res.*, **11**: 165-170.

Lakshmi N. and Nalini, P. (1989). Tertiary trisomic in *Capsicum annuum*. Cytologia, **54**: 395-399.

Laxmi, V. and Gupta, M.N. (1983). Ethyl methane sulphonate induced variability in *Trigonella foenum-graecum* L. J. Indian. Bot. Soc., **62**: 305-311.

Laxmi, V., Singh, R.B., Singh, B.D. and Singh, R.M. (1975). Meiotic anomalies induced by gamma rays and ethyl methane sulphonate treatments in pearl millet. *Indian J. Exp. Biol.*, **13**: 465-467.

Mehetre, S.S., Deshmukh, R.B. and Rodge, R.D. (1990). Effect of gamma radiations on morphological attributes in the M_1

generation of mungbean (*Vigna radiata* L. Wilczek). *Legume Research,* **13**: 14-152.

Meshram, L.D. and Hume, B.D. (1984). Rdiation induced meiotic irregularities in chilli (*Capsicum annuum* L.); Persp. Cytology and Genetics., **4**: 235-238.

Meshram, L.D. and Patil, A.N. (1986). Cytological effect of dimethyl sulphate (DMS), ethyl methane sulphonate (EMS), and acrid juice of mango (AJM) in chilli (*Capsicum annuum* L.) PKV-Research-journal, **10**: 88-92.

Meshram. L.D., Narkhede, M.N. and Deshmukh, N.Y. (1981). Spontaneous multiple translocations in *Capsicum annuum* L. cytologia, **46**: 75-80.

Mick, A.J., Mustafa, G. and Soomro, A.M. (1985). Mutation breeding in rice. SABARAO. Newsl., **3**: 121-126.

Mitra, P.K. and Bhowmik, G. (1996). Cytological abnormalities in *Nigella sativa* induced by gamma rays and EMS. *J. Cytol. Genet.,* **31**: 205-215.

Narang, K. and Prakash, G. (1983). Effect of gamma radiations on seed germination and seedling growth of some cucurbits, Acta. Bot. Indica. **11**: 36-42.

Natrajan, A.T. and Upadhayay (1964). Localised chromosome breakage induced by ethyl methane sulphonate and hydroxyl amine in *Vicia faba* L. *Chromosoma,* **15:** 156-159.

Nerker, Y.S. (1977). Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and nitrosomethyl urea in *Lathyrus sativus. Ind. J. Genet.,* **37**: 137-141.

Novak, F. and Betlach, J. (1970). Meiotic irregularities in pollen sterile sweet pepper. (*Capsicum annuum* L.) Cytologia, **85**: 385-243.

Pagliarini, M.S. (1990). Meiotic behaviour and pollen fertility in *Alptenia coridifolia* (Aizoacea). Caryologia, **43**: 157-162.

Pal, B.P., Ramanujam, S. and Joshi, A.B. (1941). colchicines induced polyploidy in crop plants II. Chilli (*Capsicum annuum* L.) Indian J. Genet., **1**: 28-40.

Parakash, N.S., Laxmi, N. and Harini, I. (1988). Cytological effects of agricultural chemicals II. Effects of fungicides "Bavistin" and "Deltan" on Chilli (*Capsicum annuum* L.) Cytologia, **53**: 709-715.

Permjit, K. and Grover, I.S. (1985). Cytological effect of some organophosphorus pesticides effects II. *Cytologia,* **50**: 199-211.

Potdukhe, N.R. and Narkhede, M.N. (2002). Induced mutations in pigeon pea (*Cajanus cajan* (L.) Millsp.). *J. Nuclear Agric. Biol,* **31**: 41-46.

Raghuvanshi, S.S. and Joshi, S.S. (1964). Cytomorphological studies on the colchicoploids of *Capsicum frutescence* L. cytologia, **29**: 61-78.

Raghuwanshi, R.K. and Singh, D. (1982). Induced mutagenesis in *Capsicum annuum* L. II. Effects of Single and combined mutagenic treatments on habit and fruit. Curr. Sci., **51**: 235-237.

Raja Rao, K.G. and Kumar, O.A. (1983). Cytogenetics of a spontaneous desyneptic mutant in Chillies (*Capsicum annuum* L.) Cytologia, **48**: 195-199.

Ramanna, M.S. (1974). The origin of unreduced microspores due to aberrant cytokinesis in meiocytes of potato and its genetic significance. *Euphytica,* **23**: 20-23.

Ramesh, D.V. and Reddi, T.V.V. (2002). induced morphological variability among three genotypes of rice. *J. Cytol. Genet.,* **3**: 115-120.

Rao, G.M. and Rao, V.M. (1983). Mutagenic efficiency and factors of effectiveness of physical and chemical mutagens in rice. *Cytologia,* **48:** 427-436.

Rao, N.B. and Laxmi, V. (1980). Gamma rays induced meiotic abnormalities in *Capsicum annuum* L. Caryalogia, **33**: 509-518.

Rao, R.Y.V., Rao, M.V.M., Ravindra Babu, V. Ramakumar, P.V. and Subramanyam, D. (1989). Efficiency and effectiveness of physical and chemical mutagens in M_2 generation of chilli (*Capsicum annuum* L.). J. Nuclear Agric. Bio., **20**: 255-259.

Reddy, S.S. and Rao, G.M. (1981). Cytogenetic effects of agricultural chemicals I. Effects of insecticides "BHC and Navacron" on chromosomal mechanism in relation to yield and yield components in chilli *(Capsicum annuum L.)* Cytologia, **46**: 699-707.

Reddy, S.S. and Rao, G.M. (1982a) Cytogenetic effects of agricultural chemical II. Effects of herbicides "Lasso and basagran" on chromosomal mechanism in relation to yield and yield components in chilli (*Capsicum annuum* L.) Cytologiya, **47**: 257-267.

Reddy, V.R.K. and Annadurai, M. (1992). Cytological effects of different mutagens in lentil *(Lens culinaries* Medik). *Cytologia*, **57**: 213-216.

Rees, H.(1955) Genotypic control of chromosomes behaviour in rye. I: Inbred lines. Heredity, **9:** 93-116.

Restaino, F (1983). Pepper bracthytic forms (*Capsicum annuum* L.) obtained through induced mutagenesis. Genetica-Agraria, **37**: 204-205.

Roy, R.R., Sinha, B.M.B. and Thakur, G.K. (1971). Irradiation studies in *Cucumis sativus. J. Cytol. Genet.*, **6**: 128-135.

Sadanandam, A. and Subhash, K. (1985). Induced multiple aneuploid in *Capsicum annuum*. Cytologia, **50**: 125-128.

Sadanandam, A. and Subhash. K. (1984). Effect of chemical mutagens on chiasma frequency in *Capsicum annuum* L. Cytologia, **49**: 415-419.

Saha, A. and Datta, A.K. (2002). Gamma rays induced reciprocal translocation in black cumin (*Nigella sativa* L.). *Cytologia*, **67**: 389-396.

Saylor, L.G. and Smith, B.N. (1966). Meiotic irregularities in species of inter-specific hybrids in *Pisum. Am. J. Bot.*, **53**: 453-468.

Sharma, A.K. and Mukherjee, R.N. (1955). Induction of chromosomal abnormalities through chemical in pollen mother cells of *Allium cepa. Genet. Iber.*, **7**: 57-64.

Sharma, B. (1986). Increasing the efficiency of mutagenesis for micromutations by early generation selection. *Indian J. Genet.*, **46**: 88-100.

Sharma, S.K. and Sharma, B. (1981a). Induced chlorophyll mutations in lentil. *Indian J. Genet.*, **41**: 328-333.

Sharma, V., Kumar, G. and Kumar, R. (2004). Comparative mutagenecity of gamma rays and EMS in *Cicer arietinum* L. *J. Cytol. Genet.*, **5**: 21-26.

Singh, A.K. (2003). Effect of doses of gamma rays and EMS on germination and plant survival in mungbean (*Vigna radiata* L. Wilczek). *Int. J. Mendel*, **20**: 99-102.

Singh, A.K. and Chaudhary, B.R. (2005). γ-rays induced chromosome anomalies in two morphologically distinct varieties of *Capsicum annuum* L. The Nucleus, **48**: 80-84.

Singh, D., Singh R.M. and Singh, J. (1989). Investigation on induced macromutants in lentil (*Lens culinaris* Medik). *Z. pflanzenzucht*, **83**: 315-320.

Singh, R.B. and Gupta, P.K. (2004). Cytological variations induced by EMS in *Clitoria ternatea* L. *Int.J. Mendel,* **21**: 75-77.

Singh, R.B., Singh, B.D., Laxmi, V. and Singh, R.M. (1977). Meiotic behaviour of spontaneous and mutagen induced partial desyneptic plnts in Pearl millet. Cytologia, **42**: 41-47.

Singh, R.K. and Gupta, S.N. (1988). Abnormal meiosis in *Capsicum annuum.* Indian J. Genet., **48**: 1-4.

Singh, R.R., (1987). Presoaking and effects of ethyl methane sulphonate concentrations in diploid and autoteraploid fenugreak. J. Cytol. Genet., **2**: 106-110.

Singh, V.P., Singh, M. and Lal, J.P. (2000). Gamma rays and EMS induced genetic variability for quantitative taits in urdbean (*Vigna mungo* L. Hepper). Indian J. genet., **60**: 89-96.

Sinha S.S.N. (1967). Radiation studies in *Lens culinaris.* Ph.D. Thesis University of London.

Sinha, R.P and Joshi, M.G. (1986). polygenic variation in hexaploid triticale in M_3 generation. *J. Nuclear Agri. Biol.,* **15**: 170-174.

Sinha, S.S.N. and Godward, M.B.F. (1972a). Radiation studies in *Lens culinaris. Indian J. Genet.,* **32**: 331-339.

Soheir, E., Antointee, H. and Atif, H. (1989). Cytological effect of herbicide Garlon-4 on root mitosis in *Allium cepa. Cytologia,* **54**: 465-472.

Solanki, I.S. and Sharma, B. (1999). Induction and exploitation of polygenic variability in lentil (*Lens culnaris* Medik). Indian J. Genet., **53**: 79-86.

Soodan, A.S. and Wafai, B.A. (1987). Spontaneous occurrence of cytomixis during microsporogenesis in almond (*Prunus*

amygdalus Batsch.) and peach (*P. persica* Batsch.). *Cytologia,* **52:** 361-364.

Sparrow, A.H. and Evans, H.J. (1961). Nuclear factors affecting radiosensitivity. I. The influence of nuclear size and structure, chromosome complement and DNA content. *Broookhaven Symp Biol.,* **12**: 76-100.

Srivastava, A. and Singh, V.P. (1993). Induced polygenic variability in pigeonpea (*Cajanus cajan* L. Millsp.). *J. Indian Bot. Soc.,* **72**: 281-284.

Srivastava, P.K. and Raina, S.N. (1981). Cytogenetics of *Tephrosia* II. Mutagenic effects of single pre and post irradiation treatments with EMS and MMS in *Tephrosia purpurea.* Cytologia, **46**: 709-721.

Stadler, L.J. (1928). Mutation in barley induced by x-ray and radium science, **69**: 186-187.

Subramanian, D. (1980). Effect of gamma radiation in *vigna.* Indian J. Genet., **40**: 187-194.

Takats S.T. (1959) Chromatin extrusion and DNA transfer during microsporogenesis. Chromosoma, **10:** 430-453

Tarar, J.L. and Dnyansagar, V.R. (1983). Gamma rays and EMS induced mitotic abnormalities in *Turnera ulmifolia* L. var. augustifolia Wild. *Cytologia,* **48**: 459-465.

Thoday, J.M. (1951). The effect of ionizing radiations on the broad bean root. IV. Chromosome breakage and .lethality of ionizing radiations to the meristem. *Brit. J. Radio.,* **24**: 572-576.

Waghmare, V.N. and Mehra, R.B. (2000). Induced genetic variability for quantitative characters in grasspea (*Lathyrus sativus* L.) Indian J. Genet., **60**: 81-87.

Wani, A.A. and Anis M. (2004). Effect of physical and chemical mutagens on various quantitative traits in chickpea (*Cicer arietinum* L.). *J. Nuclear Agric. Biol.*, **33**: 114-118.

Wani, M.R., Khan, S. and Praveen, S. (2004). Mutagenic effects of EMS in lentil (*Lens culinaris* Medik). *Int. J. Mendel,* **21**: 27-28.

Zeerak, N.A. (1991). Cytogenetical effect of gamma rays and ethyl methane sulphonate in brinjal *(Solanum melongena* L.*). Cytologia*, **56**: 187-193.